ASSOCIATION OF *Fusarium moniliforme* WITH SEED VIGOR THROUGHOUT DEVELOPMENT OF TWO ENDOSPERM MUTANTS OF SWEET CORN GROWN UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

BY

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Abstract of Dissertation Presented to the Graduate Council
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ASSOCIATION OF Fusarium moniliforme WITH SEED VIGOR
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The problem of poor seed vigor of shrunken-2 (sh2), a high-sugar endosperm mutant of sweet corn, was examined in comparison with the standard sugary (su) mutant during seed development in the field and greenhouse. Differences in seed structure and composition were determined and related to seed leakage and vigor. The interaction of Fusarium moniliforme, a seed-borne fungal pathogen, with sh2 and su during development and the effect this pathogen had on seed viability and vigor were investigated.

Seed development of sh2, as determined by kernel moisture and dry weight accumulation, appeared to be slower than su, leaving this genotype more susceptible to damage by pests and the environment. Germination and seedling growth of greenhouse-grown sh2 seeds under optimum conditions was greater than field-grown sh2 and comparable to field- and greenhouse-grown su when seeds were older than 26 days

Under cold soil test conditions, greenhouse-grown sh2 seeds had significantly greater emergance than field-grown sh2 when 38 days and older

Mature sh2 seeds had a greater imbibition rate than su for the first 60 hours of germination because of small seed size, high sugar to starch ratio, and thinner seed protective layers. After 22 days post-pollination, sh2 seeds leaked significantly more electrolytes than su seeds. No differences in carbohydrate and sugar leakage were observed between genotypes at maturity. Even though mature sh2 seeds contained less carbohydrate reserves than su, respiration rates of imbibing seeds indicated that these reserves were adequate for germination.

Kernals of sh2 became heavily infected with Fusarium moniliforme in the field earlier than su. Entrence of the pathogen was achieved through the tip cap, small cracks in the pericarp, or by appressorial formation. Further penetration into the endosperm and embryo was noted with maturity. Seed infection adversely affected viability and vigor of sh2 germinated under optimum and cold soil conditions. Radicle growth of excised embryos of sh2 and su was inhibited when exposed to Fusarium moniliforme toxin. Poor seed vigor of sh2 was related to Fusarium moniliforme infection, but this was not the primary factor involved.

INTRODUCTION

In order to obtain maximum yields and profits, growers must plant with seeds of high viability and vigor. To assure this high quality, the seed producer needs to harvest seeds when physiologically mature. One commonly used indicator of maturity is low seed moisture content, which is also important for storability. Environmental conditions sometimes hinder the attainment of full maturity. Short growing seasons in northern regions, together with seed damage caused by freezing temperature, often make the decision when to harvest seeds a difficult one for the seed producer.

Optimum plant stand is needed by the grower for highest economical returns. The increasing usages of precision seeding and mechanical harvesting have brought a greater awareness of seed germination and plant stand. Demand for high-quality seeds reflects this awareness by both growers and seed producers. Accurate and reproducible tests for viability and especially vigor are needed to assure the quality of the seeds.

Many factors can adversely affect plant stand. One of the more serious of these factors is seed-borne pathogens. These pathogens not only can cause seed and seedling rot but may also lower seed quality without killing the seed. Plant stand can be reduced and uniformity can be diminished, resulting in decreased yields and higher production costs.

In addition, seed-borne pathogens can be transmitted to the growing crop, seriously increasing disease spread with economic losses likely.

Many crops have cultivars with excellent horticultural qualities that are not commercially acceptable because of one undesirable trait or another. Sweet corn cultivars containing the shrunken-2 (sh2) gene, such as 'Florida Sweet', have shown promise commercially due to their high sugar retention after harvest. Growers and seed producers have not readily accepted these cultivars because of their poor germination and seedling vigor. The high sugar content of the seeds may increase their susceptibility to fungal attack, particularly by Fusarium moniliforme. High-sugar cultivars could substantially replace the standard sugary (su) cultivars now grown if these problems could be overcome.

The studies herein investigated the relationship of production environment during seed development with vigor of isogenic lines of sh2 and su sweet corn measured under several test conditions. The effects of their seed structure and composition on leakage and vigor and how the environment during seed development influenced these factors were also explored. Finally, the interaction of Fusarium moniliforme with sh2 and su during development and the effects of this pathogen on subsequent seed viability and vigor were determined.

CHAPTER I
LITERATURE REVIEW

Factors Affecting Germination and Vigor of Seeds

Maturation

In order for a fertilized ovule to develop into a fully mature seed, many essential processes are involved and certain factors play important roles in these processes. Adequate supplies of water, mineral nutrients, and light are necessary for the plant to supply the food reserves stored in the seed and for the seed to attain its final size. Typically, three stages can be distinguished in the development of the seed after pollination: development of the embryo, accumulation of food reserves, and the ripening or dessication of the seed (Thomson, 1979).

After sexual fusion, the growth of the embryo is slow at first but then begins to accelerate. A period of rapid cell division occurs resulting in the attainment of the final cell number of the embryo rather early in its development. Any subsequent increase in mass is the result of cell expansion and the accompanying deposition of starch, protein, and lipid. Generally, the moisture content of the seed throughout this process is about 80%.

Insufficient supplies of water, nutrients, or light to the mother plant during development of the embryo can exhibit a detrimental affect on seed production and quality. Drought conditions during the flowering stage of cowpeas substantially reduced yields (Turk et al., 1980).

Severe moisture during either vegetative or reproductive growth significantly reduced seed yields of lettuce (Izzeldin et al., 1980). This stress resulted in the lowest average number of seeds per seed head and seed heads per plant. However, average seed weights were the highest and these seeds produced the most vigorous and least abnormal seedlings. A 43% increase in abnormal embryosacs occurred in ears from corn plants that had been severely drought stressed during embryosac formation (Moss and Downey, 1971). In addition, hermephrodite spikelets were commonly observed. Silking was delayed for 13 days and grain yield was significantly reduced, even though adequate pollen was supplied. Chotena et al. (1980) found that moisture stress at silking significantly reduced both yield and seed quality.

During the period of accumulation of food reserves, the dry weight of the seed increases threefold or more, and the moisture content falls to about 50%. In most seeds, any increase in the size of the embryo is due to cell enlargement and not cell division. At the end of this stage, the seed is structurally complete (Thomson, 1979). The major events that comprise the development of non-endospermic seeds, such as legumes, are illustrated in Figure 1-1.

In corn, an endospermic seed, quantities of RNA and DNA increase in a parallel fashion in the endosperm until cell division ceases at about 28 days post anthesis, which is roughly halfway through seed formation (Duke, 1975, Ingle et al., 1964). Sugars reach a maximum in the endosperm just before the end of cell division. Starch accumulation then begins at the expense of sugars, reaching its maximum level at about 46 days. Endosperm protein increases during cell division, but undergoes a second phase of accumulation around 40 days post anthesis, which

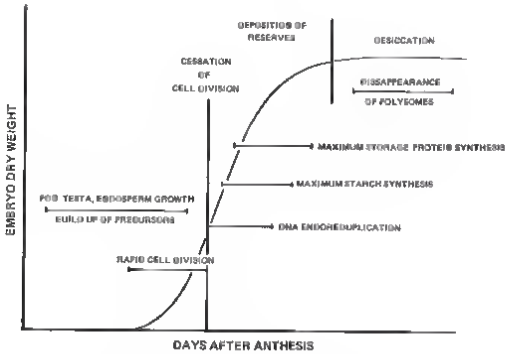


Figure 1-1 Diagrammatic representation of legume embryogenesis

SOURCE: Dure (1975).

coincides with deposition of storage protein in the aleurone layer. RNA content decreases in the endosperm during the period of rapid starch synthesis (30-46 days) while protein, RNA, and DNA build up continuously in the embryo during this 46-day period. Acid invertase activity, thought necessary for the transport of sucrose into the developing kernel, decreased from 23 to 37 days post anthesis, then increased sharply from 37 to 39 days followed by a slow decrease from 39 to 46 days (Fullerton and Svec, 1976).

An interesting hypothesis has been proposed by Tsai et al. (1978). They suggested that the proteins zein and glutelin serve as a nitrogen sink in corn kernels to regulate the movement of photosynthates into kernels. Sucrose translocation was greatest in genotypes with the highest capacity to deposit nitrogenous compounds as zein and glutalin in the kernel (Tsai et al., 1980). These proteins appear to serve as a functional nitrogen sink which increases with additional available nitrogen fertilizer. Thus, more sucrose is transported into the kernel, resulting in increased kernel weight and grain yield.

Environmental stress conditions can adversely affect the plant during the filling stage. Drought during pod filling of cowpeas substantially reduced yields by lower pod density and smaller seed (Turk et al., 1980). Soil moisture stress 2 weeks after silking in corn was associated with a significant increase in the incidence of stalk rot symptoms (Chotana et al., 1980). Corn plants with rotted stalks had more kernels than genetically identical neighboring plants with healthy stalks (Dodd, 1980a). This result could be explained by the photosynthetic stress-translocation balance concept proposed by Dodd (1977). The greater number of kernels on a particular plant causes the roots to

starve due to the demands of grain fill. The roots then rot, which predisposes the plant to stalk rot organisms (Dodd, 1980a, b).

In the ripening phase, the seed essentially dries out. There is little or no increase in the material content and the dry weight remains constant. The moisture content falls to somewhere between 10 and 20%. An abscission layer is formed which cuts off the connection with the mother plant. The time required for this stage is very dependent on weather conditions (Thomson, 1979).

Changes in cell fine structure during maturation are not destructive changes caused by excessive loss of water, but indicate physiological changes which may be necessary to prepare cells to withstand desiccation (Klein and Pollock, 1968). Howell et al. (1969) determined that respiration of ripening soybean seed was closely correlated with seed moisture content and, to some extent, temperature. Kollöffel (1970) found that during maturation of pea seed the activity of certain mitochondrial enzymes is reduced.

The determination of physiological maturity of seeds is necessary in order to harvest the best seed possible. In soybean, the seed stops importing assimilates and is physiologically mature when it is completely yellow (TeKrony et al., 1979). The respiration rate declined rapidly as the seed coat began to turn yellow and reached a low level when the seed was completely yellow at a moisture content of 55 to 60%. Crookston and Hill (1978) felt that initiation of seed shrinkage and the loss of green color of pods were consistent indicators of physiological maturity in soybeans as measured by maximum seed dry weight. In corn, a black closing layer develops in the placental region at maturity. Its appearance coincides with the achievement of maximum kernel dry weight

(Daynard and Duncan, 1969) and can be used as a visual indicator of physiological maturity (Daynard, 1972). Kernal moisture declined significantly during balck layar development (Kench and Shaw, 1971). Maximum kernel dry weight or black layar davalopment was positively correlated with maximum shoot and root dry weight upon germination (Knittla and Burris, 1976)

The degree of maturation as determined by seed moisture content or dry weight can directly affect viability. Obenderf et al. (1980) concluded that tha capahity for germination of soybean is developed before the seed reaches maximum dry weight. Matthews (1973) suggested that cotyladons and embryonic axes of pea seeds were only abla to withstand enhanced drying after removal from tha plant if the seed moisture content had already begun to decline while the seed was still on tha plant. Yield and quality of rice seed increased rapidly as seed moisture decreasad balow 43% (Delke et al., 1969). Garmination and speed of emergence were greatest when the seeds were harvested below 20% moisture.

Date of harvest is used as another indicator of seed maturity. However, performance of seed harvested too aarly can ba lass than desirabla. Knittla and Burris (1976) found that both shoot and root dry weight of corn were highly dependent on dete of harvest although germination percentagas were high throughout development. Knehler et al. (1934) datarmined that seedling vigor and field stand of corn were much bettar when harvasted in tha more mature stages than in the milk and late milk steges. Seedlings produced by immature seeds were more susceptible to seadling disaases. Matthews (1973) showed that tha younger pea seeds were at tha time of harvest, the lower their percentage

viability and the greater their percentage mortality in soil. At low soil temperatures, large differences were found in germination and stand of corn between seed harvested at 10-day intervals (Rush and Neal, 1961). In general, stands improved with an increase in seed maturity. Seed harvested after a frost gave significantly lower germination and stand under cold test conditions than unfrosted seed. Green weight of corn seedling tops from frozen immature seed was generally reduced and there was a larger percentage of weak seedlings than in unfrozen immature seed (Rossman, 1949). Freshly-harvested seed may be more sensitive to temperature during artificial drying when high seed moisture content is related to immaturity (Nellist and Hughes, 1973). The drying rate is regulated in part by hydrophilic compounds in the endosperm of the corn kernel (Ness and Crane, 1970b).

Delaying harvest past physiological maturity can reduce viability and vigor of seed. Soybean seed vigor declined rapidly, reaching levels that were significantly less than those at harvest maturity (less than 14% moisture content) within 4 to 39 days after harvest maturity (TeKrony et al., 1980). Mondragon and Potts (1974) determined that soybean seed harvested 4 weeks after physiological maturity from plants that were 50% shaded deteriorated at a much slower rate than those from unshaded plots. This difference was due to a more stable microenvironment surrounding the shaded plants. The exposure of mature soybean seed to alternate drying and wetting under field conditions resulted in reduced seed quality (Moore, 1971). Initial embryo destruction was largely seed coat related and was caused by rapid and differential absorption of water by localized tissues. Alexander and Hinson (1973) noted that the longer mature soybean seed remained in the field, the

greater the percentage of infected seed and the lower the germination. Delayed harvest resulted in decreases in viability and vigor and increases in seed-borne fungi in cotton (Roncaderi et al., 1971) and soybean (Ellis et al., 1976a, Ellis and Sinclair, 1976, Wilcox et al., 1974).

Imbibition and Leakage

Many seeds placed in an adequate amount of water under optimum conditions for germination exhibit a triphasic pattern of water uptake (Bewley and Black, 1978). Initial uptake of water in the first phase (true imbibition) is a consequence of the matric forces of the cell walls and cell contents of the seed. This phase occurs equally well in dead and living tissues and is, therefore, independent of the metabolic activity of the seed, although metabolism commences rapidly as a result of this hydration. The second phase is the lag period of water uptake, when the matric and osmotic potentials are high. In this phase, a period of active metabolism is noted in preparation for germination in non-dormant seeds or inertia in dead seeds. Respiration in germinating soybean seeds shifts from an alternate pathway (cyanide-insensitive), which is needed during the earliest stages of germination, to a cyanide-sensitive pathway, which predominates during the rest of germination (Yentur and Leopold, 1976). Embryos and their axes are capable of synthesizing proteins and RNA soon after tissue hydration. However, synthesis of RNA is not an essential prelude to the synthesis of certain proteins (Bewley, 1979). Finally, the third phase of water uptake is associated only with visible germination and subsequent growth along with the mobilization of stored reserves.

The initial pattern of water uptake is characterized in peas and many seeds by a sharp front separating wet and dry portions of the seed, continued swelling as water reaches new areas, and an increase in water content of the wetted areas (Waggoner and Parlange, 1976). Different parts of a seed, particularly a larger seed, increase in water content at different rates. When the water content of dent corn seeds reaches 75%, the water content of the embryo on a dry weight basis is 261% but that of the remainder of the seed is only 50% (Blacklow, 1972).

Certain inherent properties of the seed, such as seed size, structure, composition, and permeability of seed coats, determine the rate of imbibition. Small, flat corn seed had a faster rate of water uptake during the initial stages of germination while large, round seeds had the poorest seed vigor (Shieh and McDonald, 1980). Extensive physical changes occur in the first few minutes of water entry into the seed. Initial wetting of soybean seeds caused a release of absorbed gases which swelled the seed (Parrish and Leopold, 1977). Mitochondria became active and stable through assembly of protein into the membranes during imbibition of pea seeds (Sato and Asahi, 1975). Ultrastructurally, convoluted cell walls straightened out and organelles and lipid bodies rounded out upon exposure to water (Buttrose, 1973). The plasma membrane became relatively intact and continuous, endoplasmic reticulum extensively appeared, and mitochondria regained their shape and function after a 20 minute period of imbibition (Webster and Leopold, 1977). Compositional differences in the endosperm of wheat grains determined rate of water penetration (Brash and Milner, 1959). Radial and transverse cracks occurred only in hard vitreous endosperm in advance of water movement through the kernels. The cracks were due to

stresses between wet and dry portions. These cracks facilitatad the movement of water.

The seed coat and related protective structure directly influance the rate of water uptake in all seeds. In wheat, the seed coat or testa is tha layer offering the greatest resistance to water entry (Hinton, 1955). Lima bean seed coats can permit the seed to effectively avoid injury at low temperature and moisture stress (Pollock and Toole, 1966). When pea seed coats were removed, rapid imbibition occurred resulting in seed injury (Larson, 1968). A rapid rate of water uptake apparently caused differential swelling and cracking in the cotyledons of beans (McCollum, 1953). More than 85% of tha seeds of 22 cultivars of soybeans exhibited cotyledon cracks when imbibed in water at 22° C for 12 hours (Sorrells and Pappelis, 1976). This cracking leads to lower seed germination and vigor.

Water uptake may not occur evenly over the whole surface of an intact seed. Tha seed coats of pea seeds are differentially permeable and entry of solute into a whole seed may be exclusively through the micropyle. In addition, germination was affected by tha surface area of a seed in contact with water (Manohar and Heydecker, 1964). Entry of water into tha seed coat of hard seeds of Albizia lophantha is controlled by a small strophiolar plug next to tha hilum which is lost by eruption after heating (Dell, 1980). In cotton seed, water enters by an opening in the palisade layer at the chalazal end. This opening is made impervious by a sealed chalazal cap in hard seed. Hot water treatment (80° C) destroys this barrier (Christiansen and Moore, 1959).

The degree of lignification, thickness, and presence of phenolic compounds affect the permeability of the seed coat. In the papilionata

legumes, the impermeability to water is not imposed by waxy cuticles but by the suberized regions of the malpighian cells (Ballard, 1973). Staining with safranin and fast green indicated that the cuticle, macrosclerids, and inner integument of crownvetch seeds were lignified and responsible for hardseededness (McKee at al , 1977). Lignin comprised about 15% of the total seed coat weight for colored lima bean seeds, but only about 5% of the seed coat weight of white seeds (Kannenburg and Allard, 1964). White-seeded beans were more permeable than dark-seeded ones (Morris at al , 1968). Colored snap bean seeds had greater seed coat dry weight and thickness than white seeds. In addition, the excised colored seed coats were less permeable to water in response to an osmotic gradient than white seed coats (Wyatt, 1977). The seed coat of Pisum elatius is normally impermeable to water. However, when seeds are dried in the absence of oxygen their coats become totally permeable (Marbach and Mayer, 1974). This change may be due to oxidation of phenolic compounds in seed coats through catalysis of catechol oxidase in the presence of oxygen during dehydration of the seeds.

Environmental conditions during exposure to water, such as temperature, play important roles in the process of water uptake. Blacklow (1972) developed a mathematical equation describing imbibition by corn seeds in which imbibition temperature was a major factor. Seed injury can occur when the imbibition temperatures are above or below optimum. Extensive soybean cotyledon cracking was detected within 45 minutes imbibition at 50° C and after 5 hours at 10° C (Sorrells and Pappelis, 1976). After 12 hours, up to 83% of the cotyledons had cracked, regardless of temperature. McCollum (1953) noted that rate of water uptake

increased with temperature from 10 to 30° C but cotyledon cracking decreased.

The early imbibitional stage is critical in a seed's sensitivity to low temperature. When the chilling period was given from the moment cotton seeds began to imbibe, there was practically no growth upon transfer to optimum temperature. Seeds that were first allowed to imbibe warm water for 4 hours before the cold showed no reduction in growth (Christiansen, 1968). Excised embryonic axes of lima bean exhibited reduced growth if they were imbibed at low temperatures. Preliminary exposure to water at 25° C for as little as 10 minutes was sufficient to minimize the effects of chilling (Pollock and Toole, 1966). Low-vigor seeds were the most sensitive to this type of injury.

Many factors have been proposed and investigated to explain chilling injury in imbibing seeds. Stewart and Dewley (1980) noted that protein synthesis and levels of polyunsaturated acids were reduced in soybean seeds after an initial one hour cold treatment. Leopold and Musgrave (1979) found that chilling injury involved a major reduction in the cytochrome pathway in whole axes and cotyledons of soybeans and an engagement of the alternative pathway of respiration in cotyledon tissue. Possible differences were observed in soybean mitochondrial membranes, degree of mitochondrial integrity, and mitochondrial enzyme complement between imbibition at 10 and 23° C (Duke et al., 1977). The enzyme NADP-isocitrate dehydrogenase may limit mitochondrial respiration at low temperature. In corn, the effects of imbibitional chilling did not appear to be related to energy metabolism as measured by oxygen uptake, ATP content, adenylate energy charge, or activity of mitochondria (Cohn and Obendorf, 1976). Work by Bramlage et al. (1978) on soybeans

indicated that low temperature interferes with normal membrane reorganization during imbibition, probably by modifying the physical state of membrane phospholipids. When pea seeds were soaked in cold water, death was thought to be caused by a sudden inrush of water which disrupts the subcellular organization and membranes of a proportion of seeds predisposed to injury (Perry and Harrison, 1970). Chilling injury may be due more to rapid uptake of cold water than simply to entry of cold water (Tully et al., 1981).

The response of imbibing seeds to temperature is greatly accentuated by their moisture content. Injury was dependent upon the initial seed moisture content prior to imbibitional chilling in lima bean (Pollock, 1969), garden bean (Pollock and Manalo, 1970), sorghum (Phillips and Yeongman, 1971), soybean (Hobbs and Obendorf, 1972; Obendorf and Hobbs, 1970; Sorrells and Pappelis, 1976), and corn (Cal and Obendorf, 1972, Conn and Obendorf, 1976, Obendorf et al., 1971). Seeds at low moisture content (5 to 8%) were injured while seeds at 13 to 20% initial moisture exhibited no symptoms of injury during germination. Thus, it is dry seeds that suffer chilling injury during early imbibition, once they become hydrated they are much less sensitive (Simon, 1974).

The soil can supply water to the germinating seed at a rate equal to or higher than the capacity of the seed to absorb it. The amount of water absorbed by the seed and the rate of germination depend on the internal potential adaptability of the seed and on the actual moisture potential at the seed-soil interface (Hadas, 1970). Minimum moisture contents for germination of corn and soybean are 30.5 and 50%, respectively. At 25° C, a soil should have a moisture tension of not

more than 12.5 atmospheres for corn and 5.6 atmospheres for soybean to germinate (Hunter and Erickson, 1952). Water diffusivity of soybean and corn seed germinating in silt loam soil at four moisture contents ranging from wilting percentage to field capacity was approximately equal at each soil moisture content (Phillips, 1968). More adverse effects of increasing osmotic pressure (drought conditions) were noted on weak-vigor than on strong-vigor corn seed lots, and on the shoot than on primary root elongation (Parmar and Moore, 1968). In a study by Shaykewich and Williams (1971), it was found that resistance to water absorption in rapeseed was very high during the early stages of imbibition. This resistance decreased greatly as seed water content increased. They also suggested that hydraulic conductivity in soil may have an influence on imbibition rate in the late stages of water absorption.

Desiccation of the germinating seed may occur in the field after imbibition has started. Generally, no harmful effects are noted if desiccation proceeds before cell division and enlargement has commenced. However, once embryo growth is apparent, embryo damage of some sort usually results (Gerrie and Drennan, 1971). Delteur and Jacqmard (1974) demonstrated that drought sensitivity in corn seed was related to the onset of nuclear DNA synthesis and genome duplication. Rehydration of drought-sensitive corn embryos causes breakdown in plasma and nuclear membranes which leads to loss of cellular compartmentalization. In addition, the chromatin remains condensed and has lost its function of genetic regulation (Crevecoeur et al., 1976).

When seeds are immersed in water or planted in moist soil a variety of substances leak out of them, such as organic acids, sugars, and

amino acids. Normally, the seed coat restricts or regulates water uptake by acting as a barrier to diffusion. Cracking or removal of the seed coat increases the velocity of water uptake and solute leakage. Seed coats of white-seeded beans cracked more readily than black-seeded cultivars before emergence (Prasad and Weigle, 1976). This cracking tendency increases leakage from white-seeded beans and makes them more susceptible to attack by soil pathogens. Leachates from pea seeds with seed coats removed always contained more solute than leachates from intact seeds (Larson, 1968). Duke and Kakefuda (1981) found that seeds with intact seed coats leak detectable levels of enzymes with high specific activities while seeds without seed coats leak high levels of many intracellular enzymes. They suggested that legume seeds with broken or missing seed coats are damaged by cellular rupture during imbibition. Matthews and Rogerson (1976) felt that differences in leaching of solutes from different seed lots of peas were associated with the condition of the embryos and not the seed coats. In addition, these differences were related to the ability of the embryos to retain solutes rather than their initial solute content. Simon (1974) believes that the seed coat acts as a barrier slowing down the external release of solutes as well as reducing the extent of leakage from the embryo itself.

Generally, the rate of leakage declines dramatically even during the first few minutes of imbibition. After 30 or 60 minutes of imbibition, leakage declines to a slow rate (Larson, 1968, Simon, 1974, Simon and Raja Harun, 1972). Eyster (1940) suggested that alterations in the normal differential permeability of cell membranes in the seeds with subsequent loss of essential cell constituents should be reason for

further seed injury. Simon and Raja Harun (1972) proposed that, as seeds dry out in the course of development, cell membranes lose their integrity. When such dry seeds are allowed to imbibe water, there is a short period, before membrane integrity is reestablished, during which solutes can leak out of the cells. In seeds, leakage slows down as the outer cells, which are hydrated before those inside, become less leaky while the inner ones begin to leak but contribute less due to a longer path of diffusion (Simon, 1974). A popular view of the molecular mechanism of leakage states that, as cells hydrate, the membranes rapidly change from a relatively porous hexagonal state to a lamellar condition which halts free diffusion of solutes (Parrish and Leopold, 1977, Simon, 1974). However, McKersie and Stinson (1980) examined a phospholipid-water system from seeds of birdsfoot trefoil using x-ray diffraction and found these 'membranes' to be exclusively lamellar, even at 9% water content. Another possibility is that undissociated organic acids increase membrane permeability by changing the lipid components of the membranes (Jackson and St. John, 1980).

Species vary in the amount of exudate released by germinating seeds. Temperature, as well as moisture content, influences the quantity of seed exudate. Greater rates of leakage in eight types of seeds indicated a prominent increase in permeability of the plasmalemma in the 30 to 35° C range (Hendricks and Taylorson, 1976). Permeable corn seeds, as indicated by a high concentration of solid material leaching during a soaking period, were more susceptible to cold-test conditions (Tatum, 1954). The majority of carbohydrate was exuded from pea seeds during the first 18 hours of incubation at 22 or 30° C, but at 10° C significant carbohydrate exudation persisted for about 48 hours (Short and

Lacy, 1976). Soybean seeds are sensitive to low temperature during imbibition, with leakage the greatest at temperatures below 15° C (Bramlage et al., 1978, Hobbs and Obendorf, 1972, Leopold, 1980, Leopold and Musgrave, 1979). Increasing the moisture level of soybean seeds to 20% and embryos to 35% markedly reduces their cold sensitivity (Bramlage et al., 1978, Knypl, 1979). Leakage was very low if pea embryos already had a water content of 30% or more (Simon and Wiebe, 1975). Dehydration of wild oat embryos imbibed for 24 hours or of birdsfoot trefoil seeds imbibed for 18 hours resulted in decreased germination and vigor and increased cytoplasmic leakage during rehydration (McKersie and Tomes, 1980). Using birdsfoot trefoil seeds, McKersie and Stinson (1980) found that desiccation-tolerant (imbibed less than 12 hours) seeds leaked relatively low quantities of all solutes while desiccation-sensitive (imbibed for 24 hours) seeds leaked higher levels upon reimbibition.

Quantities of total water-soluble sugars were the same in both high- and low-quality soybean seed lots prior to imbibition. However, following imbibition, high-quality seeds were better able to mobilize and utilize these energy reserves for germination and early seedling growth (Wahab and Harris, 1971). More electrolytes exuded from dead and low-vigor pea seeds than from high-vigor seeds (Perry and Harrison, 1970). Loss of membrane integrity appears to be a common phenomenon of mechanically injured, stored, and low-vigor seeds. McDonald (1975) believes that measurements of solute leakage such as conductivity, or other techniques emphasizing membrane structure, could provide reliable estimates of seed vigor. Highly significant correlations have been shown between electrical conductivity and/or soluble carbohydrate content of seed steep water with seed vigor and field emergence in peas

(Bedford, 1974, Matthews and Bradneck, 1967, Perry, 1970), soybeans (Knypl, 1979, Yaklich et al., 1979), rapeseed (Takeyanagi and Murakami, 1968), and corn (Barjak and Villiers, 1972, Holaday, 1964).

Seed lots that leak most heavily are the most prone to soil mortality. Wrinkle-seeded varieties of peas leaked more and were more susceptible to attack by Pythium than smooth-seeded varieties (Flentje and Saksena, 1964). Conditions which favored high amounts of nutrient exudation generally corresponded with conditions which favored high levels of pathogen spore germination around seeds leading to high amounts of seedling decay (Short and Lacy, 1976). A direct causal relationship was suggested between amounts of carbohydrate released from seeds and disease incidence. This relationship has been confirmed in peas (Perry, 1973) and soybeans (Keeling, 1974). At less than optimum soil temperatures, germinating seeds and seedlings remained in the zone of seed exudation for a greater period of time, leaving them susceptible to attack by microorganisms (Schroth et al., 1966). Substances leaking out of seeds into the soil stimulated the germination and growth of fungal pathogens in the soil (Schroth and Cook, 1964, Short and Lacy, 1974). On the other hand, extracts from seed coats of black-seeded cultivars of bean contained phenolic compounds that inhibited growth of Rhizoctonia solani (Prasad and Weigle, 1976).

Seed Pathogens

Many plant pathogens are disseminated almost wholly or predominately by seed. Some seed-borne pathogens have sources of inoculum other than seed which are important or other agents of dissemination which prevail, such as soil, wind, water, insects, and nematodes

These types of pathogens are harder to control than strictly seed-transmitted ones. The losses caused by these pathogens are staggering and cover the cereals, legumes, oil crops, vegetables, fiber crops, and others. Neergaard (1977) reviewed the major crops, the seed-borne pathogens affecting them, and their losses in detail.

A better knowledge of the mechanics of seed transmission may lead to better methods of controlling diseases. Attention must be given to the growth stages of the seed crop, weather conditions, time and paths of infection, affected parts of the seed or fruit, and the conditions and course of infection in the plant developing from the infected seed. Eight principal types of disease cycles and infection courses are discussed by Neergaard (1977). Pathogens exhibiting these types of disease cycles are truly seed-transmitted. Establishment of a pathogen in, on, or with the seed implies that the pathogen is seed-borne, and may or may not be seed-transmitted. Seed-borne pathogens either contaminate (infest) or infect seeds.

There are considerable differences between different kinds of seeds in regard to frequency of transmission of any one pathogen, and in the number of different pathogens so transmitted. In some plant families seed transmission of pathogens predominates, in others it is rare. Many seed-borne pathogens are frequently transmitted in Gramineae and Leguminosae. The seed-borne smut and ergot fungi, as well as a great variety of Drechslera species, are characteristic of the mycoflora of Gramineae seeds. Viruses, bacteria, and a group of fungi that produce 'anthracnose' diseases are commonly occurring pathogens in Leguminosae. Another family, the Cruciferae, transmit many of the Alternaria diseases. A comprehensive annotated list of seed-borne diseases,

arranged according to hosts, has been published by Noble and Richardson (1968).

An essential feature in relation to infection and transmission of pathogens is the relative size of the embryo within the body of the seed. Differences in shape and position of the embryo in the seed may also be relevant. Infection of the embryo, in particular of the thick nutritious cotyledons, of Leguminosae by fungi and bacteria is remarkably common, and the prevalence of seed-borne diseases may be largely assigned to the dominance of the embryo in the seed. Pathogens have ample access to the cotyledon in maturing seeds, and transmission is highly favored by localization in the cotyledons. In Gramineae, on the other hand, the embryo is small and chances for embryo infection more limited. If the embryo does become infected, as it may at earlier stages in the development of the caryopsis, it is usually killed resulting in seed abortion and often in considerable yield losses (Neergaard, 1977).

Viruses

Viruses, bacteria, and fungi, the three main types of pathogens, are transmitted by and can affect seed quality in some way. The least common pathogen in seeds, viruses, are covered extensively and summarized by Bennett (1969) and Phatek (1974). Although most viruses are not seed-borne, the number of viruses for which seed transmission has been recognized is steadily growing, and is rather frequent in some genera of plants. This occurrence holds true for certain genera of leguminous plants (Leguminosae), stone fruits (Amygdalaceae), and composites (Compositae). Many leguminous crops are susceptible to

viruses, some harbor a considerable range of them, many are seed-transmitted, and most of them embryo-borne. Most seed-transmitted viruses are able to invade the host systemically and establish a compatible relationship with the tissues of the embryo allowing both virus and host to perpetuate themselves. However, there are viruses that do invade the embryo, but they do not survive as the seed matures. In addition, some highly infective seed-borne viruses are carried in the seed coat (Neergaard, 1977).

Bacteria

Bacteria are commonly carried on seed and any bacterial plant pathogen may potentially be seed-transmitted. Lack of endospores limits their survival in soil and normal crop rotation will control many of them. Seed transmission is of particular importance in cultivated annuals such as bean, soybean, pea, cucumber, cabbage, cotton, tomato, and cereals. The direct pathogenic effect of bacteria on seeds may include seed rot, seed abortion, seed discoloration, and/or 'slime disease' (Neergaard, 1977).

Location of bacteria within the seed will differ with host and pathogen involved. Many bacteria are carried on the surface of the seed. Some important examples are Xanthomonas vesicatoria on tomato, Corynebacterium flaccumfaciens on bean, Pseudomonas lachrymans on cucumber, and Xanthomonas campestris on cabbage. Bacteria causing vascular or systemic infections are frequently found in the seed coat or other tissues of the seed. Seed-borne bacteria are often referred to as 'deep-seated' but are usually confined to the seed coat. For instance, Pseudomonas phaseolicola and Xanthomonas phaseoli in bean are found in

tha hilum region of tha saed, into which they penetrate from the vascular system through tha funiculus (Neergaard, 1977). In soybean, Bacillus smbtilis was found in tha parenchyma and hour-glass cell layers of seed coats (Tenne et al., 1977). Surface-borne bacteria may keep alive for a limited period of time only, perhaps one or two years, whereas bacteria harbored within saed tissues may liva longar.

The only major seed-transmitted bacterial pathogen of corn is Erwinie stewartii, Stewart's becteriel wilt of corn. Rand and Cash (1921) isolatad tha bacterium from tha tissues of the endosperm. Ivanoff (1933) demonstrated that it occurs intracellularly in the chalazal tissues and between the innermost row of cells of the chalazal region and the marginal layer of the andosparm and in the andosparm itself. Infected seeds are important in transporting tha bacteria to diffarent areas, but probably are unimportant in overwintering. It is likely that infected seed provides an infection source for spread of the pathogen by flea baetlas (Pepper, 1967).

Fungi

Most of the seed-transmitted pathogens are fungi. Some classes or genera are frequent in saed, others occur only occasionally or parhaps not at all. Some are easily detected, others do occur but cannot ba revealad by conventional testing procedures. The extent to which fungi occur in seeds depends on their capability to surviva under tha extreme dry conditions of seed. Hydrophilic fungi, such as downy mildew, are unable to produce, or produca very few, resting structures. Tha survival of the fungi depends on the longevity of mycelium within tissues of seed. Xerophilic fungi are capabla of producing resting

structures that can withstand dessication, such as chlamydospores, conidia, and sclerotia. Some examples of these fungi are Alternaria, Drechslera, Cercospora, and the covered smuts of cereals.

The direct impact of fungi on seed is considerable. Many fungi are serious parasites of seed primordia and maturing seeds. They can reduce yields of seed both quantitatively and qualitatively. Other fungi, including saprophytes and very weak parasites, may lower the quality of seeds by causing discoloration, seriously depreciating the commercial value of seeds graded for consumption. Types of diseases and disorders encountered include seed abortion, shrunken seeds, reduced seed size, seed rot, sclerotization or stromatization of seed, necrosis, discoloration, reduction or elimination of germination capacity, and/or physiological alterations in seed. A complete list of seed-borne fungi and their hosts has been compiled by Neergaard (1977).

Seed transmission of fungi. Over the years, a variety of testing procedures were developed in order to determine seed transmission of pathogens. Seed health testing is based primarily on culture tests that are accurate, reproducible, quick, inexpensive, and easy to use. These tests involve the seed, the pathogen, and incubation. Major factors involved in the condition of the seed, such as injury, vigor, age, and pretreatment, are important to the outcome of any test. The kind, amount, distribution, and vigor of inoculum of the pathogen at the time of testing are principal objectives. Culture medium, temperature, humidity, light, and time all influence the incubation stage of a test. Leach (1967) and Chuleiprasit et al. (1974) recommended using an alternating cycle of 12 hours exposure to near-ultraviolet light and 12 hours

of darkness throughout the period of incubation. Interfungal antagonism can be eliminated by using a selective culture medium (De Tempe and Limonard, 1973). Slow-growing fungi, such as Septoria nodorum in wheat seed, are determined in this way (Luke et al., 1977). Factors of incubation are reviewed more extensively by Neergaard (1973).

Detection of seed-borne fungi. Cursory examination of seeds or their parent plants for physical evidence of infection is not as accurate as culture or incubation tests. Filipowicz (1976) found that pea seeds attacked by Ascochyte and Fusarium spp. do not always reveal stains on the pods. Corn seed from ears judged disease-free gave rise to high percentages of Fusarium moniliforme or Diplodia zeae (Miller, 1952). Internal infection of corn seed by Fusarium moniliforme and Cephalosporium acremonium occurred without external symptoms (Christensen and Wilcoxson, 1966). Drechslera maydis did not produce disease on non-corn hosts from which it was isolated in low percentages (Fatima et al., 1974).

The five major types of culture or incubation tests are based on (1) growth of pathogen, (2) growth of seedling and pathogen, (3) growth of seedling and development of symptoms, (4) growth of seedling beyond the seedling stage and development of symptoms, and (5) indicator plant tests (Neergaard, 1973). Mycelial growth rate can be determined by the standard agar plate test. The growth of seedling and pathogen are measured in the standard blotter test. A modification of this test involves deep-freezing sometime during incubation to either kill the seed or seedling. A seedling-symptom test is conducted in soil or sand and is useful in determining seed treatments and seed vigor. The fourth

type of test is called a growing-on test and is used extensively in quarantine procedures. Inoculation of indicator plants can detect and identify seed-borne viruses and determine viability of pathogens.

Sensitivity comparisons of some of these tests emphasize differences due to seed type, pathogen, and worker involved. Lacicowa et al. (1975) estimated more precisely the percentage of bean seeds infected by Colletotrichum lindemuthianum and Fusarium spp. by determining the number of infected seedlings grown in sand. Comparable percentages of rice seed infection by Drechslera oryzae were recorded on blotter tests and potted seedlings (Aulakh et al., 1974). After 15 days the blotter test gave much higher counts for Fusarium moniliforme from rice seeds than the agar plate method (Nath et al., 1970). Identification was made of species of Fusarium associated with different kinds of seeds on the basis of colony characters on the seed in the blotter test. Kulik (1971a) detected Drechslera maydis in corn seeds with a blotter method. Mannerucci and Gambogi (1976) found that 7 days incubation of pea seeds in towels at 20° C in the dark was the most sensitive method for Ascochyta pisi. The deep-freezing method was selected as the most suitable for routine seed health testing by Singh et al. (1974) and Mathur et al. (1975). Kulik (1973) explored the possibility of detecting certain pathogenic fungi in seeds by their production of unique metabolites when plated on selective media.

Incidence of seed-borne fungi. Infection percentages as determined with the classic incubation methods are often insufficient for evaluating a seed-borne infection. A reduction in germination and field emergence of soybeans was not noted until recovery of Sclerotinia

sclerotiorum was more than 25% (Nicholson et al., 1972). Richardson (1970) figured a multiplication of 2 8 of symptoms seen in the germination test would give an estimate of the amount of total infection Using this method, Alternaria brassicicola, A. brassicae, and Plenodomus lingam occurred in 41, 10, and 4%, respectively, of samples of seeds of Brassica spp. The work of De Tempe (1968) showed that in certain cases a slight infection in a seed is most dangerous for the growing crop in the field, while in other cases a slight infection in a seed will be below the threshold level for pathogenicity. The quantity as well as the type of pathogens per seed needs to be ascertained.

Seed-borne fungi may provide sufficient inoculum to infect the resulting crop. Lorenzini et al. (1975) found that while Fusarium oxysporum f. sp. pisi was present in low percentages of pea seeds (0.5% or less) damage to plants did occur. The number of spinach seeds infected with Verticillium dahliae in commercial lots was rather low, but high enough to serve as a first source of infection in subsequent crops (Van der Spek, 1973). Holbert et al. (1924) concluded that many corn seeds have only small infections and are most difficult to detect. Such seed may produce plants that are severely infected with root and stalk rot. Singh et al. (1967) proved that the internally seed-borne nature of corn downy mildews was important in transmission in India. Cephalosporium acremonium is largely seed-borne, and the disease arises each year from infected seed (Reddy and Holbert, 1924). Seed transmission of crazy top of corn was demonstrated by Ullstrup (1952), but seed infection is probably of little practical consequence in disseminating the disease. Infected bean seed could provide soil-borne inoculum in the form of sclerotia as a result of colonization by Whetzelinia

sclerotiorum with little opportunity for spread from infected plants (Steadman, 1975). Corn lines with Texas male-sterile cytoplasm had a greater incidence and transmission of Helminthosporium maydis and Fusarium moniliforme (Boothroyd, 1971, Crosier and Braverman, 1971; Warmke and Schenck, 1971).

Geographic area influences the incidance of a particular seed-borne pathogen. Fusarium moniliforme was the most prevalent species isolated from corn seed samples from 21 states (Manns and Adams, 1921). Occurrence of internally-borne fungi of soybean seed lots from five states was influenced more by growing area than by planting date, harvesting data, or harvesting method (Nicholson and Sinclair, 1971). Each of three species of Fusarium on corn was maximal in a specific geographic area in South Africa (Marasas et al., 1979). Fusarium moniliforme predominated in a subtropical area, F. moniliforme var. subglutinans in the most temperate area, and F. graminearum in the area with an intermediate climate.

Fungal interactions may affect incidence of a seed-borne pathogen. Helminthosporium maydis ear rot predisposed corn seed to fungal invasion and aflatoxin contamination (Dompnik, 1972). The predominant fungus on damaged seeds was Fusarium moniliforme. McGee et al. (1980) obtained significant negative correlations between the amount of soybean seed infected by Cercospora kikuchii and that infected by Phomopsis, Fusarium, or Alternaria spp. In addition, a significant positive correlation occurred between seed infection by Phomopsis and Fusarium spp.

Insect transmission. The importance of insect transmission of plant pathogens, including parasitic fungi, is well known. Slight injury

of corn ears at the tip from earworms caused marked increases in infection by some fungi (Koehler, 1942). The most common pathogen isolated in injuries caused by insects is Fusarium moniliforme (Koehler, 1960). Other examples of insect-transmitted seed-borne fungi are Diplodia zeae and Fusarium graminearum, for which the corn earworm and European corn borer provide entries for infection and aid in rapid spread within corn plants (Christensen and Wilcoxson, 1966, Taylor, 1952). A reduction in corn earworm infestation by Smeltzer (1955) resulted in a 20% reduction in corn kernel damage by Fusarium moniliforme. Picnic beetles may also serve as a vector for Fusarium spp. on corn (Windels et al., 1976). Insect injuries are important in other seeds, such as soybeans, for fungal entrance (Kilpatrick, 1957). Stinkbug injury was not necessary for fungal infection of soybean seed but did adversely affect seed development (Kilpatrick and Hartwig, 1955).

Mechanical transmission. Numerous methods have been used to artificially inoculate corn with ear-rotting fungi. Some are aerially disseminated while others simulate insect damage. Loesch et al. (1976) sprayed silks with inoculum 10 days after they emerged. Using this technique, Diplodia zeae caused the most ear damage. A high incidence of infection was observed when ears were inoculated by spraying silks 6 to 19 days past pollination (Warren, 1975). Inoculations made after 22 days resulted in only small amounts of kernel rot. Ullstrup (1970) obtained good results using either the silk spray or insertion of toothpicks overgrown with mycelium into ear shoots prior to silking and without injuring the cob. Application of inoculum of Gibberella zeae as a spray 1 to 2 weeks after full silk resulted in the highest amount of ear rot,

while Diplodia maydis was less specific. Fusarium moniliforme ear rot severity was greater when ears were inoculated 10 days after silking by an ear-puncture method compared with a silk-spray technique (Anlye et al., 1980). An interesting method was used by Boling et al. (1963). They shot BB-pellets covered with Fusarium moniliforme spores into ears at 10 to 20 days after silking. This technique, along with tip injection at 10 days, produced the most infection.

Time of infection. The invasion of fungi at different stages in the development of the seed is of great importance to the fate of the seed, which often is killed at the early stages. If invasion takes place when the seed is nearing full maturity, then only the seed coat or pericarp is invaded. During the later stages of seed development, the morphological barriers within the seed may hinder penetration of hyphae into deeper layers (Neergaard, 1977). According to Seltzer (1930, cited by Neergaard, 1977), Fusarium culmorum, scab or Fusarium blight, produces the heaviest infection on rye during flowering, leading to incomplete development of the grain. If the infection takes place during grain maturation, then the grain is less affected. At any point in between, infection leads to damage of gradually decreasing effect. Wilson et al. (1945) found a similar occurrence in seed of Lolium perenne infected by blind seed disease, Gloeotinia temulenta. If infection occurred before the pericarp had dried, then the embryo was killed. The germination capacity of the seed was not effected by infection after the pericarp dried. The mycelium of Acroconidiella eschscholtziae were incapable of penetrating into the embryo when seeds of California poppy attained a certain degree of maturity (Davis, 1952).

Quantitative and qualitative losses resulting from infection of rice by Helminthosporium oryzae were most severe when rice was inoculated in the flowering and milk stages (Fazli and Schroeder, 1966) The number of developed kernels decreased as a result of inoculation Corn ears inoculated with Fusarium moniliforme about 1 to 2 weeks after silking produced the greatest amount of ear rot and seed infection (Boling et al., 1963, Gulya et al., 1980, Warren, 1978).

Delayed harvest of mature seeds increases the time of infection by many pathogens. The percentage of soybean seed infection was lowest prior to maturity and gradually increased through and after maturity (Kilpatrick, 1957). Fungi isolated from immature soybean plants increased 55% from 4 to 7 weeks after planting (Kmetz et al., 1974). The number of soybean seed infected with fungi, such as Diaporthe phaseolorum var. sojae, Alternaria and Phomopsis spp., increased and seed germination decreased with time of harvest after maturity (Athow and Laviolette, 1973; Ellis et al., 1976a, Kmetz et al , 1974, Wilcox et al., 1974) Time of harvest did not influence infection with Cercospora kikuchii (Athow and Laviolette, 1973, Wilcox et al , 1974). The delay in harvest adversely effected early-maturing more than late-maturing cultivars (Alexander and Hinsen, 1973, Kilpatrick, 1957, Wilcox et al., 1974).

Location of Infection. The outer structure of seeds, such as appendages and sculpture of seed coat or pericarp, is often important for establishment of infection or in providing conditions for contamination The bracts in beat seeds are susceptible to infection by Phoma betae and Cercospora beticola, and the glumes of cereals assist in

harboring infections of major pathogens such as Drechslera, smuts, and downy mildews (Neergaard, 1977). An asparagus seed surface is rough, and fungal spores of Fusarium moniliforme and F. oxysporum f. sp. asparagi are trapped in natural crevices or insect tunnels (Inglis, 1980). Infestation occurs when the seed is extracted from the berries.

The seed coat is the common site of infection of most seed-transmitted Fungi Imperfecti. In a healthy soybean seed, the seed coat consists of a cuticle, palisade, hour-glass, and parenchyma cell layers. The hour-glass cell layer is the site of initial colonization by Cercospora kikuchii, Colletotrichum truncatum, Diaporthe phaseolorum var. sojae, and Phomopsis spp. (Balkan et al., 1976, Ellis et al., 1975, Ilyas et al., 1975, Schneider et al., 1974, Sinclair, 1976, 1977, 1978). Infections, established as dormant mycelium or other resting structures, have been demonstrated for Verticillium dahliae in spinach and beet (Van der Spek, 1972, 1973), Phoma lingam in cabbage (Jacobsen and Williams, 1971), Rhizoctonia bataticola in okra (Vir and Gaur, 1970), Ascochyta rabiei in chickpea (Maden et al., 1975), Fusarium oxysporum f. carthami in safflower (Klisiewicz, 1963), Verticillium albo-atrum in sunflower (Sackston and Martens, 1959), Helminthosporium oryzae in rice (Fazli and Schroeder, 1966), Cladosporium spp. in barley and wheat (Flannigan, 1974), and Rhizoctonia solani in tomato and pepper (Baker, 1947). Internally seed-borne fungi were located within the seed coat tissues of barley (Warnock and Preece, 1971) and bean (Balkan et al., 1976, Ellis et al., 1976c). Sclerospora sorghi was confined in mature corn kernels to the pericarp and pedicel (Jones et al., 1972). The embryo and endosperm were protected from the fungus by the aleurone layer which appeared to serve as a barrier.

Deeper penetration of e fungus results in a more massive infection, which ceuses more direct damage to the embryo of the seed Weston (1920) found mycelium of Sclerospora philippinensis, Philippine downy mildew, in the endosperm and seed coat of apperently healthy corn kernels. Miller (1952) established thet Diplodie zeae first invedes the embryo of corn end then penetrates into the endesperm and pericarp The pathogen of crezy top of corn, Sclerospora macrospora, occars within the embryo es well es in the endosperm (Ullstrup, 1952). Downy mildew of corn is borne in the embryo, mostly in the upper part of the plumule and coleoptile (Singh et el , 1967). Inter- ond intracellular mycelium of Rhizoctenie soleni were observed by Baker (1947) in the endosperm es well as the embryo of Capsicum frutescens Both superficiel end deep infections were equelly potent in the transmission of Ascochyta rabiei in chickpea (Naden et al , 1975) end Alternaria zinniae in Zinnia (Gamboqi et al , 1976) Fusarium moniliforme was frequently present in both endosperm and embryo of sorghum (Mathur et el , 1975).

The thick nutritious cotyledons of leguminons seeds often become more or less deeply infected They may be regarded es the main site for e number of fungi infecting the embryo of legumes Some examples are Colletotrichum lindemuthienum in bean (Zeumeyer and Thomas, 1957), C truncatum in soybean (Tiffani, 1951), and Ascochyta pisi in pea (Dekker, 1957, cited by Neergaard, 1977). In 75% of pee seeds infected, the fungus had penetrated deeper then the seed coat and 40% of the epicotyls were infected

Path of infection Seed primordium or maturing seed may be infected either directly from the mother plant or by transmission

from outside. Infection directly from the mother plant may be introduced through the flower or fruit stalk (pedicel, peduncle) and the seed stalk (funiculus), or directly from the seed surface (integuments). The raphe is a continuation of the funiculus, often fused with the seed coat, and contains vascular strands which provide additional chances for transmission of vascular-infecting fungi. Vascularization is more extensive in fruit coats than in seeds. Bean, cotton, cucurbits, flax, pea, and tomato have seeds with raphe and are known to transmit vascular-infecting fungi such as Fusarium oxysporum, Verticillium albo-atrum, and V. dahliae (Neergaard, 1977).

Rudolph and Harrison (1945) isolated Fusarium moniliforme, F. oxysporum, and F. equiseti from vascular bundles in all parts of cotton, including the internal tissues of the seed. However, there was no indication that the infected plants were diseased, or that the seed was damaged by the presence of the fungus. The frequency of Diaporthe phaseolorum var. sojae and Phomopsis spp. was higher in lower portions of soybean plants than in the upper portions, higher in stems than in green pods, and higher in green pods than in seed (Kmetz et al., 1974). Percentages of infected seed increased with time of harvest after maturity. Fusarium oxysporum f. sp. lycopersici, Fusarium wilt of tomato, produces vascular mycosis and penetrates through the fruit stalk and funiculus into the seed (Baker, 1952). Seeds of beet and spinach are colonized by Verticillium dahliae invading from the vascular system of the mother plant (Van der Spek, 1972). Mycelium of Diplodia zeae appeared to grow from the corn cob at the butt end through the grain attachment and into the embryo (Miller, 1952). Kucharek and Kommedahl

(1966) felt that systemic infection of corn kernels by Fusarium moniliforme was insignificant in the transmission of this pathogen.

Infection from outside may be introduced through the stigma, the ovary wall or pericarp, and the flower or fruit stalk, and later through the seed coat. A pathogen may penetrate several of these parts of the seed, and in turn infect them (Neergaard, 1977). Acroconidiella eschscholtziae on California poppy can penetrate into the capsule from infected petals through the point of attachment without producing visible symptoms on the capsule (Davis, 1952). The mycelium may also penetrate in turn the fruit stalk, capsule, and seed coat. Rhizoctonia solani entered from the pericarp of Capsicum frutescens into the placenta and further through the funiculus into the embryo and endosperm (Baker, 1947).

Many fungi may penetrate through the tissues of the pods directly into the seeds. Some examples are Colletotrichum lindemuthianum on bean, Ascochyta pisi on pea, Alternaria spp. on crucifers (Neergaard, 1977), Leptosphaeria maculans on rape and oilseed radish (Petrie and Vanterpool, 1974), and Diaporthe phaseolorum var. sojae and Alternaria spp. on soybeans (Athow and Laviolette, 1973; Kilpatrick, 1957). Seed damage increased towards the bottom of plants (Hepperly and Sinclair, 1980) and in pods in contact with soil (Ellis et al., 1976d). Soybean seeds with a lower level of impermeability had higher levels of infection (Miranda et al., 1980).

Natural modes of entrance of fungi into corn ears were investigated by Koehler (1942). Fusarium moniliforme generally entered in the region of the silks and the kernels became contaminated in contact with

the silks. The infection then spread to the pedicels, vascular cylinder, and finally the shank. A large percentage of infection with Cephalosporium acremonium took place in the same manner. Infection down the ear by way of the glumes could also occur. Gibberella zeae infection progressed from the tip and through the silks. Diplodia zeae and Nigrospora spp. caused infection by entering at both the tip and butt ends of ears. Fungal penetration at the butt was largely the result of local infections on the shank. Internal kernel infection in sound-appearing kernels generally did not become established until the ears were approaching maturity.

Effects on seed and seedling. As stated previously, seed-borne fungi may affect the seed and/or subsequent seedling in various ways. One such way is to reduce seed yield. Fusarium head blight, caused by Fusarium moniliforme, significantly reduced the weight and size of sorghum kernels, with a resultant 3% decrease in seed yield (Castor and Frederiksen, 1980).

Discoloration of seeds is a very important degrading factor. In seeds for planting, such disorders may indicate that seed-transmitted fungi are present, although this may not be the case. In seeds for consumption or industrial use, it may be a general indication of poor quality. Cercospora kikuchii causes a purple stain in soybeans and reduces seedling emergence somewhat (Wilcox and Abney, 1973). Rice quality is related to the degree of discoloration, which in turn reflects the extent of mycelial spread of Helminthosporium oryzae within the seed (Fazli and Schroeder, 1966). Discolored or small shriveled bean seeds accounted for all of the isolations of Whetzelinia

sclerotiorum from roots of healthy plants and from most lots from diseased plants (Steadman, 1975). White streaks on the pericarp of corn kernels were caused by Fusarium moniliforme, Cephalosporium acremonium, or Nigrospora spp. invasion of the pericarp. The white color was due to the disintegration of cells which caused them to lose their transparency and take on a chalky appearance (Koehler, 1942).

Many seed-borne fungi produce seed rot either in the crop or during germination. Complete descriptions of ear and kernel rots of corn can be found in "A Compendium of Corn Diseases" (Shurtleff, 1977). A severely infected seed will cause pre-emergence death of a seedling while a slightly infected seed will carry the disease into the growing crop (De Tempe, 1969). Deeply seated infections in Zinnia seed caused severe infections to occur on the seedling as soon as growth began leading to pre-emergence death (Gambogi et al., 1976). Fusarium solani and Phyllosticte phaseolina caused 30 and 20% bean seed rotting, respectively, after artificial seed inoculations. When inoculum was added into soil before planting, 36 and 10% pre-emergence rotting occurred and there was 35 and 15% post-emergence blight as well (Gupte and Saharan, 1973). Wrinkled-seeded pea varieties were more liable to fail in emergence due to seed rot by Fusarium spp. than smooth-seeded varieties (Lambhate and Ghide, 1976). Work by Madesen and Hodges (1980) suggested a combination of direct toxicity to germinating creeping red fescue seed and an enhancement of the pathogenicity of seed-rotting Drechslera sorokiniana and Curvularia geniculata induced by nitrogen-containing compounds.

Seed-rotting fungi can produce superficial necroses in the seed. In leguminous seeds anthracnose fungi, Colletotrichum spp., as well as

Ascochyta spp., often penetrate into the fleshy cotyledons, producing conspicuous necrotic lesions in seeds of bean, soybean, pea, chickpea, cowpea, and other hosts (Neergaard, 1977).

Because of seed-borne fungi, poor germination and emergence result in lowered plant stands. Necroses or more deeply penetrating rots in seeds reduce the viability of seeds, their longevity in storage, and their emergence in the field. In addition, infections which do not cause rot are known to lower the viability of seeds (Neergaard, 1977). Adverse field conditions accentuate seed infection and reduce viability and vigor. Significant correlations between seed infection and germination and/or emergence were recorded in corn with Drechslera maydis (Kulik, 1971b), in bean (Ellis et al., 1976d), in sorghum with Fusarium moniliforme (Nethur et al., 1975), and in soybean with Diaporthe phaseolorum var. sojae (Ellis et al., 1974, Nicholson et al., 1972, Tenne et al., 1974, Wallen and Cuddy, 1960, Wallen and Seaman, 1963, Wilcox et al., 1974). Phomopsis spp. (McGee et al., 1980), Sclerotinia sclerotiorum (Nicholson et al., 1972), and Fusarium spp. (McGee et al., 1980).

When Diplodia zeae is seed-borne in corn, it enters the mesocotyl through wounds caused by the emergence of seminal roots (Christensen and Wilcoxson, 1966). Nwigwe (1974) determined that Diplodia zeae and a Phomopsis caused up to 37 and 34% losses in germination, respectively. Reddy (1933) found that corn seed lots that germinated readily at low temperatures (below 11° C) were injured very little by Nigrospora oryzae, whereas corn seed that required a higher temperature were usually severely injured. The reverse is true for Gibberella zeae and certain species of Fusarium (Dickson, 1923).

The severity of symptoms on seedlings does not always appear directly connected with the damage during germination (Lorenzini et al., 1975). Even seedling symptoms may not be related to disease development and intensity on plants (Mannerucci and Gambogi, 1976). Maude (1966) noted that 4 to 6 weeks after planting pea seeds infected with Mycosphaerella pinodes almost all the seedlings showed disease lesions at or below soil level. Pea seeds infected with Ascochyta pisi led to only 40% infection of the subsequent seedlings. Lesions occurred on the stem and first two leaves within 4 weeks after planting.

Metabolic products of seed-borne fungi, notably toxins, may adversely affect seed germination and seedling growth. The inhibition of sorghum seed germination by Colletotrichum graminicole and Fusarium moniliforme was due to extracellular, thermostable toxins (Tripathi, 1974). A seedling blight of corn was caused by F. moniliforme and the mode of action was by a heat-stable and completely water-soluble toxin which inhibits root growth (Futtrell and Kilgore, 1969; Scott and Futtrell, 1970). Symptoms of abnormality and reduced growth of uninfected corn embryos were found by Srodnik (1975) to be due to toxic metabolites of F. graminearum and F. moniliforme. Corn seeds which had germination abnormalities only on radicles or on plumules and those seeds which developed decayed and distorted plumules were recognized by Kushibiki et al. (1976) in seeds of a particular inbred line. They isolated two species of Fusarium and one species of Penicillium which seemed to cause the abnormal germination. The highest percentage of toxic F. moniliforme var. subglutinans isolates was obtained from the geographic area in which it occurred most frequently (Marasas et al., 1979). A toxin preparation from Helminthosporium maydis Race T caused

multiple changes in isolated Texas male-sterile cytoplasm corn mitochondria (Gregory et al., 1980). These changes included uncoupling of oxidative phosphorylation, stimulation of succinate and NADH respiration, inhibition of malate respiration, increased swelling, loss of matrix density, and unfolding of the inner membrane. Severe ultrastructural damage only to mitochondria was observed within 20 minutes.

Control of seed-borne fungi. Seed treatment is probably the cheapest and often the safest method of direct plant disease control. The aim of seed treatment is to prevent infection of the seedlings and the subsequent crop. The mechanism of the effect may vary, the inoculum in or on the seed may be killed directly by the treatment itself, or later during the germination of the seed and pathogen. Treatment of infected seed may thus be curative but should also protect the seeds from attack by organisms that are present in the soil (Neergaard, 1977). A considerable range of physical and chemical procedures have been brought into use for disinfection or protection of seed against pathogenic fungi. Only some of the more common or newer methods will be reviewed.

For many years chemical seed treatment consisted of coating or soaking seeds in mercuric compounds, such as Ceresan and Agrosan. These compounds provided a wide range of protection and were universally used. Seed treatment was effective against Ascochyta and Fusarium spp. in pea (Filipowicz, 1976), Rhizoctonia bataticola in okra (Vir and Gaur, 1970), various seed-borne fungi in soybean (Agrawal, 1974), and in various vegetable seeds (Sahi, 1976). In recent years there is a definite trend away from use of mercuric compounds, in view of the risk involved to

people and animals. Legislative precautions have been taken in various countries to limit or eliminate the use of these compounds (Neergaard, 1977).

Seed treatment chemicals replacing mercuric compounds include organic sulphurs (Thiram, Maneb), quinones (Spergon, Phygon), polychlorobenzenes (HCB, PCNB), heterocyclic nitrogen compounds (Captan, thiabendazole), antibiotics (streptomycin, aureofungin), and various systemic fungicides (benomyl, carboxin). The most commonly used compounds are Captan and Thiram. Treatment with Thiram increased field emergence and decreased seed-borne fungi in dry beans (Ellis at al., 1976d), peas (Short et al., 1977), and other seeds (Maude et al., 1969). Captan or Thiram effectively controlled Ascochyta pisi and Mycosphaerella pinodes in peas (Maude, 1966), and internally seed-borne fungi in bean (Ellis at al., 1976c) and soybean seeds (Ellis et al., 1975). Many internally seed-borne fungi are located in seed coat tissues and only occasionally found in embryo tissues. Captan and Thiram moved into seed coat tissues, but did not penetrate the embryo. They were effective only against fungi on or in the seed coat (Ellis at al., 1975). The antibiotic aureofungin was found to significantly reduce the infection of rice and cabbage seed by Pyricularia oryzae and Pleiochaeta lingam (Rahalkar and Neergaard, 1969). Fumigation of high quality soybean seeds with propylene oxide, formaldehyde, or hydrazoic acid eliminated most surface fungi (Schlub and Schmitthenner, 1977). However, these treatments are costly and may adversely affect seed germination and vigor.

Systemic fungicides, such as benomyl and carboxin, have recently been developed and used as seed treatments. Benomyl controls a wide

range of Fungi Imperfecti and Ascomycetes, such as Ascochyta, Colletotrichum, Fusarium, Phoma, Septoria, and Sclerotinia. Bollen and Fuchs (1970) found that most Ascomycetes and the Hyphomycetes (Blastosporae, Phialosporae, Aleurosporae) were usually highly sensitive to benomyl, whereas Oomycetes and Hyphomycetes (Porosporae, such as Drechslera and Alternaria) were resistant. The systemic carboxin has a narrow spectrum of activity, being effective mainly against Basidiomycete fungi (Edgington et al., 1966).

Deep-seated as well as infections located on the seed coat can be eradicated by systemic fungicides. Benomyl effectively controlled Ascochyta pisi, Fusarium oxysporum f. sp. pisi, and Mycosphaerella pinodes in pea (Lorenzini et al., 1975; Maude and Kyle, 1970, Yoshii, 1975), Phomopsis spp. in soybean (Bolkan et al., 1976; Ellis et al., 1975, Shertt and Sinclair, 1980), Phoma lingam in cabbage (Gabrielsen et al., 1977; Jacobsen and Williams, 1971), and Fusarium moniliforme and Cephalosporium acremonium in corn (Singh et al., 1971). Sharma et al. (1976) reported poor control of Corticium rolfsii in corn with benomyl. Phytotoxicity has been noted with benomyl in soybeans by Ellis et al. (1975). Combination of benomyl with Thiram provided better control than either compound used alone in soybeans (Nedrow and Harman, 1980) and other seeds (Mills and Wallace, 1972). Carboxin applied as a seed treatment effectively controlled smut fungi of cereals (Hansing, 1970), while carboxin plus Thiram increased seedling stands of corn seed infected with Helminthosporium maydis Race T (Lim and Kinsey, 1973). Perateluene sulfonamide eradicated seed-borne downy mildews in corn (Singh et al., 1967) and pyroxychlor controlled damping-off and root rot

of soybeans by Phytophthere megasperma var sojae (Pepavizas and Lewis, 1976).

Systemic fungicides are capable of more movement through the seed and seedling than Captan or Thiram. Benomyl penetrated the seed coat and embryo of soybean seeds and was effective against fungi in both areas (Ellis et al., 1975). Singh et al. (1971) detected benomyl in 10-day-old corn seedlings from treated seeds. Thapliyel and Sinclair (1971) used labelled and unlabelled systemic dust fungicides as seed treatments of soybean. They demonstrated that benomyl and chloroneb initially tended to localize in the cotyledons, while carboxin did not. Subsequently, chloroneb became localized in cotyledons, lower hypocotyl and roots of the seedling, carboxin was distributed uniformly throughout the seedling, with higher concentrations in the epicotyl, whereas benomyl moved only into the epicotyl. Chloroneb thus has potential for control of root and lower-stem infections, while benomyl and carboxin might be more effective against pathogens invading the epicotyl tissues.

Many seed-borne pathogens are harbored within the tissues of the seed, particularly the outer layers. Successful treatment is largely dependent on the capacity of the fungicide to penetrate into the seed. It is well known that the protective layers, when physically intact, constitute efficient barriers against entry of most organisms, as well as chemicals, into the deeper layers of the seed. Mechanical injuries provide access for increased attack of invading fungi and increased damage by toxic chemicals. Green and Minton (1980) noted that no fungicide treatment completely compensated for mechanical damage to the seed coat of cotton. The defense mechanism of pericarp and seed coat is mechanical as a physical impediment, but is also physiochemical, mainly

by being selectively permeable to chemical substances in solution (Neergaard, 1977)

The use of organic solvents, such as dichloromethane (DCM) or acetone, for incorporating fungicides into seeds can increase fungicide movement and effectiveness and overcome the seed's selective permeability. Application of the fungicide pentachloronitrobenzene (PCNB) by means of DCM proved highly effective in making pea seeds resistant to infection by Aspergillus ruber (Tao et al., 1974). Soybean seeds treated with methyl 2-benzimidazole-carbamate (MBC) or thiabendazole in DCM had decreased incidence of internally-borne Phomopsis spp. and higher germination and emergence than seeds treated with DCM alone (Ellis et al., 1976b) Infusion of pyroxychlor dissolved in acetone into soybean seed before planting controlled damping-off and root rot caused by Phytophthora megasperma var. sojae (Papavizas and Lewis, 1976) This treatment did not effect root nodulation or cause phytotoxic symptoms even after a 30 minute soak. Shortt and Sinclair (1980) treated soybean seeds with one of six contact or six systemic fungicides in acetone, DCM, or polyethyleneglycol (PEG). Fungicide activity was detected in seeds treated with the systemic benomyl, carboxin, isathane, and thiabendazole. Larger inhibition zones and a greater reduction of seed-borne Phomopsis spp. occurred when benomyl and isathene were infused with PEG than with either acetone or DCM The efficacy of any treatment depended on the fungicide and solvent-carrier combination.

Commonly, seeds of poor germination or vigor are more adversely effected by chemical seed treatment than seeds of high germination or vigor. Poor seed quality may be due to many factors, such as

physiological immaturity, mechanical damage, and high moisture content infaction by seed rot-producing fungi may provide avenues for penetration of chemicals to deeper layers of the seed, thus favoring conditions for phytotoxic effects of the chemical (Neergaard, 1977).

A non-chemical seed treatment can involve the use of a hot water soak. For many years cereal seeds have been soaked in hot water to control smut fungi. Other types of seed-borne fungi can be controlled with this treatment. Davis (1952) eliminated Heterosporium eschscholtziae from California poppy seed with a hot water treatment at 51.5° C for half an hour. Lo (1975) used a hot water treatment to reduce the percentage of fungi-contaminated vegetable seeds, but was unable to completely control seed-borne diseases. Treatment at 50° C for 10 minutes had no detrimental effect on seed germination, but longer times could reduce vigor. At high levels of seed infection, hot water treatment may be inadequate. Williams (1967) found that an epidemic of blackleg of cabbage was caused by a low percentage of Phoma lingam in a seed lot which withstood a hot water treatment of 50° C for 25 minutes. This seed lot contained 18% infected seeds. Pritchard (1974) treated popcorn seed with a mixture of steam and air for 17 minutes at 54-55° C and successfully eradicated Helminthosporium maydis from internally-infected seed.

Sodium hypochlorite can surface disinfect seeds. Lo (1973) significantly reduced seed-borne organisms on vegetable seeds with sodium hypochlorite treatment. High concentrations and long soak time reduced germination rates. Dempsey and Walker (1973) found that sodium hypochlorite was better than calcium hypochlorite at equal concentrations for surface disinfection of pepper seed. Treatment with sodium

hypochlorite increased germination and vigor of pepper (Fialdhouse and Sasser, 1975) and cyclamen seed (Anderson and Widmer, 1975). However, this stimulation may have been due to removal of surface-borne organisms detrimental to the seed.

Various natural fungal inhibitory agents have recently been demonstrated for seeds. Srivastava and Mishra (1971) found antifungal substances from seed coat leachates of Paspalum scrobiculatum and Triticum aestivum were active against Alternaria tennis. Capric acid, isolated from American elm seeds, was identified as an antifungal agent against Dutch elm disease, Ceratocystis ulmi (Doskatch et al., 1975). Volatile fungal inhibitor (VFI) tablets were effective against several seed-borne fungi (Vir and Gaur, 1971). A complex lipid was isolated from dry whole pea seeds that inhibited sporulation, but not growth, of many seed pathogens (Pflegar and Harman, 1975). Aqueous extracts of colored pea seed coats inhibited Pythium ultimum hyphal growth, but uncolored seed coats contained no fungistatic compounds (Stasz et al., 1980). Resistant pigmented pea seeds exuded greater amounts of phenols than susceptible green-seeded pea seeds (Neergaard, 1977).

Fungal interactions may provide a form of biological control of seed-borne and soil-borne fungi. Roy and Abney (1977) noted that incidence of Cleporthe phaseolorum var. sojae was reduced in pods and seeds of soybean plants inoculated at various growth stages with Cercospora kikuchii. Coating corn kernels with antagonistic microorganisms and planting them in soil inoculated with Fusarium graminearum resulted in control of seedling blight at 18-20° C (Cheng and Kommedahl, 1968). Kernels coated with either Bacillus subtilis or Chaetomium globosum gave equal results in the field as kernels treated with Captan

or Thiram. In laboratory studies, treatment of pea or radish seeds with Trichoderma hamatum protected seeds and seedlings from Pythium spp. or Rhizoctonia solani nearly as effectively as fungicide seed treatments (Harman et al., 1980).

Cultural practices, such as application of fungicides during crop growth, can reduce seed infection. Prasartsee et al. (1975) reported that seeds from soybean plants sprayed with various fungicides had significantly less internally-borne Diaporthe phaseolorum var. sojae than those from nonsprayed plants. Foliar applications of benomyl reduced the incidence of seed-borne fungi of soybeans at maturity, and partially suppressed the increase in fungi and decrease in germination associated with late harvest (Ellis et al., 1976a, Ellis and Sinclair, 1976). No significant differences between application time and rates of benomyl were found by Ellis et al. (1974). Laeffler et al. (1980) recorded the greatest improvement in soybean seed quality for a single benomyl application at the green pod stage. When wheat foliage was sprayed with two to six applications of benomyl, the number of colonies of Alternaria and Bipolaris spp. that developed on the seeds increased, while colonies of Septoria and Epicoccum spp. decreased (Luke et al., 1977).

Fungal inoculum may be inactivated by aging during the storage of seeds. However, many parasitic fungi are so persistent in the seed that they are capable of outliving their hosts. Thick-walled resting mycelium within the seed survive longer than unprotected spores on the seed surface. Moniliaceous Hyphomycetes, such as Botrytis, Cercospora, and most Fusarium spp., have more or less hyaline, thin-walled conidia and are usually short-lived. Noteworthy exceptions in this group are

Fusarium moniliforme and Cephalosporium acremonium, both of which may invade their host systemically. Dungan and Koehler (1944) noted that corn seeds still harbored viable F. moniliforme after 8 years, while C. acremonium died after 7 years. Dematiaceous Hyphomycetes, such as Drechslera and Alternaria spp., have conidia with thick walls and strong pigmentation and have been found to be quite long-lived. Neergaard (1977) summarizes many of the seed-borne fungi and their storage life.

The longevity of fungi is quite dependent on storage conditions. Lutey and Christensen (1963) found substantial reduction in percentage of field fungi, such as Alternaria, Drechslera, and Fusarium, in barley kernels kept for a few months at 14% moisture content and 20° C. After 24 weeks all Drechslera and Fusarium died out, Alternaria was reduced from 80 to 10%, and the germination capacity remained above 90%. When moisture content was reduced to 12%, the field fungi were all present in low percentages after 53 weeks of storage at 20° C. On the other hand, kernels stored at 16% moisture and 20 or 30° C were overcome by storage fungi and the germination percentage of the seed decreased rapidly. Marasas et al. (1979) determined that the mean percentage of corn seed infected by Fusarium moniliforme, F. graminearum, and F. moniliforme var. subglutinans actually increased after 8 months storage under commercial conditions.

Structure and Composition of the Corn Kernel

The kernel of corn (Zea mays L.) is a fruit composed of a thin pericarp enclosing a single seed. The pericarp is the mature ovary wall and comprises all the outer cell layers down to the seed coat (Kiesselbach, 1949; Wolf et al., 1952a). The seed coat in turn encloses

the germ and the endosperm, the three forming the seed. This type of single-seeded fruit, in which the pericarp does not open on drying to release the seed, is characteristic of the cereal grains and is known as a caryopsis (Wolf et al., 1952a).

The pericarp is the outermost structural part of the kernel except over the relatively small area at the base covered by the tip cap. Tissues of the tip cap and pericarp are continuous, forming a complete covering for the seed. A variation in thickness of the pericarp is noted and is due primarily to differences in compression over different parts of the kernel rather than to differences in the number of cell layers (Wolf et al., 1952b). The transformation of the ovary wall into the pericarp is a gradual process and requires the entire period from fertilization to maturity for its completion (Randolph, 1936). A progressive series of changes is involved, which includes growth by cell division and enlargement, disintegration and collapse of cells in certain regions, extensive lignification of cell walls, and a final compression of the entire tissue into the relatively thin protective covering of the mature caryopsis. The amount of growth involved is very significant, since the dimensions of the mature kernel are from 9 to 12 times as great as those of the mature ovary from which it developed (Randolph, 1936).

The pericarp's inner surface is in direct contact with the seed coat. The seed coat is the outermost structure of the seed itself. According to Wolf et al. (1952b), the seed coat is a thin, suberized, hyaline membrane located between the aleurone layer and the tube cells of the inner pericarp. Its exact origin has always been disputed. Whether it arises from the nucellus (Kiesselbach, 1949; Kiesselbach and

and Walker, 1952, Randolph, 1936) or as a secretion from the inner epidermis of the inner integument (Johann, 1942, Wolf et al , 1952a, b), the seed coat entirely encloses the germ and endosperm except over the basal portion of the kernel. The hilar layer, continuous with the seed coat, covers the basal portion of the kernel. Unlike the seed coat, the thick hilar layer is distinctly cellular in structure (Wolf et al , 1952b).

In all seeds, the seed coat originates directly from the integuments which cover the ovule in the early development of the seed. Generally, these integuments undergo changes to form a hard, resistant seed coat, which is often many cell layers in thickness. This is the protective covering of the mature seed. In corn, this protective function is assumed by the pericarp, the integuments degenerating until at maturity only a thin, hyaline membrane, called the seed coat, remains (Kiesselbach, 1949, Randolph, 1936, Wolf et al., 1952a).

The endosperm of corn comprises about 80% of the dry weight of the kernel (Wolf et al , 1952a) The outermost layer of the endosperm, consisting of thick-walled aleurone cells containing oil and protein bodies, is sharply differentiated from the thin-walled starchy cells that make up the rest of the endosperm. This aleurone layer forms a covering that encloses the germ and the starchy endosperm and which is interrupted only over the hilar layer at the base of the kernel. In this small area, a patch of thin-walled parenchymatous cells, distinctly different structurally from the aleurone cells but continuous with them, constitutes the outer layer of basal endosperm (Kiesselbach and Walker, 1952, Wolf et al , 1952c) This region serves for conducting food from the mother plant to the growing endosperm and then to the embryo

(Kiesselbach, 1949). Over the face of the germ, the aleurone layer constitutes the only endosperm tissue. The thick aleurone cell walls constitute an added semipermeable envelope enclosing the germ and starchy endosperm and controlling the entrance of water and solutes into the kernel (Wolf et al., 1952c). However, the aleurone layer will support a heavy growth of fungi when the seed coat is penetrated (Johann, 1935). The walls of the starchy endosperm cells are much thinner than those of the aleurone cells. Within these cells, the individual starch granules are embedded in a proteineceous matrix (Wolf et al., 1952c).

The germ or embryo, consisting of the embryonic axis and scutellum, is embedded in the lower portion of the endosperm just beneath the face of the kernel and parallel to its long axis. It comprises about 10 to 15% of the dry weight of the kernel (Wolf et al., 1952a). The water-holding capacity of the embryo is much greater than that of the endosperm surrounding it. Under conditions favorable for germination, the embryo imbibes about 60% of its weight of water as compared with only about 25% by the endosperm (Tonle, 1924).

The embryonic axis is made up of those parts of the embryo which undergo further growth and development upon germination. However, the embryonic axis only makes up less than 3% of the dry weight of the kernel (Wolf et al., 1952a). The axis consists of a primary root and a plumule joined by the mesocotyl.

The scutellum is much larger than the embryonic axis and comprises roughly 10% of the dry weight of the kernel (Wolf et al., 1952a). The scutellum encases the embryonic axis leaving only the tips of the shoot and root exposed. Only the scutellum is in contact with the endosperm tissue. Most of the scutellum is made up of storage parenchyma cells

that are heavily pitted, filled with dense protoplasm with large nuclei, and contain predominantly lipids with some starch (Kiesselbach and Weihaar, 1952, Wolf et al., 1952d). The main functions of the scutellum are in the translocation of nutrients to the developing embryonic axis during maturation and germination (Wolf et al., 1952d) and in the secretion of hydrolytic enzymes into the endosperm to break down starch (Dure, 1960b).

Endosperm Mutants of Corn

There are many genetic mutations in corn which have been shown to affect endosperm components. Several of these mutations have been shown to alter the type and quantity of carbohydrates, especially starch, in the kernels. Still others can alter the protein content in the kernels. A detailed history of many of the endosperm mutants can be found in a review by Creech (1968). However, some specific mutants will be briefly covered here.

Mertz et al. (1964) reported that the opaque-2 (o2) gene significantly increases lysine and tryptophan content of the endosperm. Normally, corn endosperm is deficient in lysine due to the predominance of the prolamin type of protein. In o2, the zein (a prolamin) content is lower but the glutelin and albumin proteins, with a higher lysine content, are raised (Bawley and Black, 1978). However, o2 hybrids germinate poorly in the field (Loesch and Bailey, 1980) and in cold tests (Gupta and Kovacs, 1974) and are more susceptible to kernel and ear rots (Loesch et al., 1976; Ullstrup, 1971; Warren, 1978).

Collins (1909) described a waxy (wx) mutant that was different from normal dent corn in sugar and starch content. Wexy mutants produce

starch that is practicelly ell amylopectin (Batas et al , 1943) Amylopectin is the major component in most corn endosperm starches, ranging from 75 to 85%, and consists of chains of α(1-4) and α(1-6)-glucosidic linkages to form a treelike structure. Amylose is the other component of corn starch and is primarily linear with α(1-4)-linked glucose residues

Two sugary genes (su and su2) were described by East and Hayes (1911) and Eryster (1934). The su mutant has higher levels of reducing sugar, two to four times more sucrose, a dramatic increase in water-soluble polysaccherides (WSP), and half as much starch as normal dent corn during early development, 16 to 28 days after pollination (Creech, 1965). This mutant has long been incorporated into commercial sweet corn hybrids. Kramer and Whistler (1949) established that the su2 gene increased the amylose content to 35% at maturity

Vineyard and Seer (1952) described amylose-extender (ae) which substantially increased the amylose content. The ae endosperm produces starch with about 50% amylose In addition, a substantial increase in sugars and reduction in starch was noted. When ae was combined with wx and du wx, a dramatic increase in sugars and reduction in starch occurred (Creech, 1965). The triple mutant ae du wx has recently been incorporated into a commercial hybrid (Garwood and Creech, 1979) Starch granules from this mutant had a significantly greater digestibility than starch granules from normal (Garwood and Vanderslice, 1980)

Cameron and Teas (1953) have shown that two other endosperm mutations, brittle-1 (bt) and brittle-2 (bt2), reduce endosperm starch substantially without the accumulation of WSP. The gene bt was first described by Maeglesdorf (1926) while bt2 was characterized by Teas and

Tees (1953). Both genes caused increases in reducing sugars and sucrose and reductions in total starch as compared to su and normal (Cameron and Tees, 1954). Whole kernels of the double mutant bt su contain about 40% as much WSP and 200% as much sucrose as the su mutant alone (Tsai and Glover, 1974). In addition, 90% of the sucrose is preserved even up to 4 days post-harvest at room temperature.

The gene shrunken-1 (sh) is an endosperm mutant that reduces total starch significantly, causing the formation of a collapsed or shrunken kernel. This mutation was first described by Hutchinson (1921). A mutant with a phenotype similar to sh was shown by Mains (1949) to be a distinct mutation from sh and was designated shrunken-2 (sh2). Less starch is stored in sh2 endosperm than in normal and su endosperms (Laughnan, 1953). Total sugars were increased tenfold over normal and fourfold over su at maturity (denting stage) with most of this increase due to sucrose. The amounts of WSP in sh2 kernels are low and similar to normal.

Laughnan (1953) concluded that the sh2 gene preceded the sh gene in the synthesis of starch and WSP. The gene sh2 apparently caused a block between the sugars and polysaccharides (Creech, 1968). Tsai and Nelsen (1966) reported that sh2 completely lacked adenosine diphosphoglucose (ADPG) pyrophosphorylase activity in both endosperm and embryo tissues 22 days after pollination. This enzyme converts glucose 1-phosphate to ADPG, a key step in starch synthesis (Figure 1-2). However, Dickinson and Preiss (1969) found that total activities of this enzyme were about 12% in sh2 and 17% in bt2 of that found in normal 22 to 30 days after pollination. With starch synthesis reduced due to low amounts of ADPG pyrophosphorylase in the endosperm but not the embryo

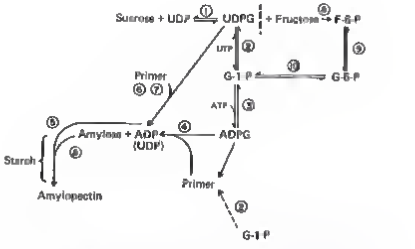


Figure 1-2 Summary of the mechanism for starch synthesis in cereals

SOURCE Bewley and Black (1978).

(Hennah and Nelson, 1975), a build-up of sugars occurs. Higher levels of sucrose and reducing sugars at all stages of development occur in sh2 and bt2 (Cameron and Teas, 1954, Creech, 1965, Creech and McArdle, 1966).

High-sugar endosperm mutants have certain favorable quality characteristics. Wann et al. (1971) evaluated the carbohydrate composition of nonisogenic su, sh2, and ae du wx hybrids at harvest and after 7 days of storage at 18° C. The sh2 and ae du wx cultivars contained from 1.5 to 2.0 times more total sugar at harvest than su cultivars. All genotypes lost sugar during storage, however, sh2 and ae du wx contained significantly more total sugar than the su cultivars after storage. The sh2 and ae du wx cultivars were low in WSP but relatively high in starch after storage. All cultivars had approximately the same amount of total carbohydrate. Moisture loss from the kernels during the storage period was significantly less in the sh2 and ae du wx cultivars. They maintained their fresh appearance longer, and were slower than su to show kernel denting.

In a study using isogenic lines, Garwood et al. (1976) showed that the ae du wx and sh2 genotypes produced high quality canned and frozen products. Kernel sucrose values on a dry weight basis in unstored ears were 36.5% for sh2, 24.8% for ae du wx, and 14.4% for su. After 96 hours of storage at 27° C, sucrose content decreased from initial levels by 83% in su, 63% in sh2, and 56% in ae du wx. In spite of these decreases, ae du wx and sh2 sucrose content after storage was almost as high as that found in unstored su ears. This fact demonstrates the superior post-harvest sugar retention for sh2 and ae du wx.

A major limiting factor to the acceptance and success of the high-sugar types by the sweet corn industry has been low seed and seedling vigor. Many of these mutants germinate and grow significantly less than su and normal even under optimum conditions (Ness and Crane, 1970a). In a study by Rowe and Garwood (1978), genotypes with the ae phenotype, including ae du wx, had poor vigor and exhibited some reduced germination and decreased seedling shoot length. In contrast, genotypes with the sh phenotype performed consistently better than the ae genotypes. Hannah and Cantliffe (1977) observed that 9 days after planting, the percent stands of two sh2 hybrids were 38 and 66%, while sh was 82% and bt-A was 92%. Germination and seedling vigor measurements showed that sh2 was significantly lower in both laboratory and field tests than su, bt, and normal (Styer et el , 1980). The seeds of sh2 hybrids are smaller, lighter, and more easily damaged than su seeds due to the higher sugar and lower starch content of the endosperm. Dry weight of sh2 and ae du wx kernels was significantly lower than su or normal (Wann, 1980). The endosperm to embryo dry weight ratio was also low in the high-sugar lines due primarily to their small endosperm.

The combination of small seed and high sugar can increase susceptibility of the seed and seedling to fungal rots. Stand losses observed with sh2 hybrids were from seed rot and pre- and post-emergence damping-off (Berger and Wolf, 1974, Pieczarka and Wolf, 1978). Isolations from seed before planting commonly yielded Rhizopus, Fusarium, and Penicillium spp., even though the seed was commercially treated with Captan and/or Thiram (Berger and Wolf, 1974). In addition to the above fungi, isolations from damped-off seedlings 7 to 14 days after emergence yielded Rhizoctonia solani and Pythium spp. Plants that were moderately

effected and still survived were stunted and remained smaller than apparently healthy plants. Stunted plants always failed to develop a marketable ear.

Several fungicidal seed treatments have improved stand and uniformity of growth in sh2 hybrids. Berger and Wolf (1974) found that a seed slurry treatment of Benlate plus Difolitan significantly improved plant stand on muck soils. Pleczarka and Wolf (1978) used the combination of Benlate plus Difolitan to effectively increase plant stand by 35% and yield by 38% when compared to the untreated control. Mixtures of Difolitan plus Dexon and Benlate plus Dexon were the best treatments for increasing plant stand on both sandy and muck soil (Cantliffe et al., 1975). Yield was improved by up to 95 crates per acre when the Difolitan plus Dexon treatment was compared to the untreated control. Only Difolitan has been cleared for limited use as a sweet corn seed treatment, thus restricting the use of these fungicides to research only. However, even the best of these seed treatments does not provide a plant stand as adequate as those obtained with routinely treated su seed. The grower must compensate by overseeding and then thinning.

The cause of low seed vigor in the high-sugar genotypes is not fully understood. It is not entirely known whether the low seed vigor is due to a smaller endosperm or whether the embryo itself is genetically inferior and incapable of exhibiting strong vigor. Wann (1980) determined that respiration rates of germinating seeds of sh2, se du wx, sn, and normal did not account for growth differences among the genotypes. He concluded that the low seed vigor in high-sugar genotypes was related to their small endosperms. The genotype of the embryo also was important in seedling vigor, but the low vigor could

not be wholly attributed to genetic inferiority of the embryo. Styer et al. (1980) concluded that adenosine triphosphate (ATP) levels in seeds of sh2 did not appear to be the limiting factor to poor vigor during the early stages of germination.

Vigor Tests

There is no one universal vigor test for all seeds. A vigor test can only measure one phase of early seedling growth. Some of the simplest means of estimating vigor are by measuring the seed's physical characteristics. For lettuce seeds, Smith et al. (1973a) and Soffer and Smith (1974) found that seed weight was more important than seed width or thickness in predicting vigor. Smith et al. (1973b) and Scaife and Jones (1970) showed a linear relationship between seed weight and emergence and yield. Seed size and protein content were significantly related to seedling vigor in wheat (Ries and Everson, 1973) and bean (Ries, 1971). Seedling emergence rate index and grain yields significantly increased with large wheat seed (Bulisani and Warner, 1980). Ferguson and Turner (1971) found that emergence and seedling survival were related to seed fullness in cotton. Sivasubramanian and Ramakrishnan (1974) proposed that seed size of groundnut, in conjunction with one or two vigor tests, could serve as an index of field emergence potential.

Physiological tests measure some aspect of germination or seedling growth under favorable or unfavorable conditions. Tests under favorable conditions involve speed of germination or rate of seedling growth as important factors (Woodstock, 1973). Measurements of seedling growth may include fresh and dry weights, height, radicle and hypocotyl lengths.

Early corn seedling growth rates may reflect seed injury caused by a variety of agents (Woodstock and Feeley, 1965). Woodstock and Combs (1964) found that shoot growth provided the best basis for predicting subsequent seedling performance. Gill and Delouche (1973) determined that first count germination percentages and seedling growth rate were the most practical, consistent and sensitive measures of progress of deterioration of stored corn seed. An 80-hour-count germination test was proposed by Mian and Coffey (1971) as an accurate vigor test for corn seed. The standard warm germination test had the highest correlation with field emergence under optimal conditions for soybeans (Polizotto, 1980) and navy bean (Suryatmane et al., 1980). Laboratory speed of germination tests have also been successfully used to predict field performance (Larsen and Isely, 1967, Perry, 1967).

Additional physiological tests emphasize placing seeds under stress conditions either prior to imbibition or during the germination process itself. The advantage of these tests is that germination remains the criterion for vigor evaluation (McDonald, 1975). The two main types of stress tests are the cold test, with or without soil, and the accelerated aging test.

The cold test is one of the oldest methods of stressing seeds for vigor evaluation. This test simulates cold and wet field conditions that occur with early spring planting. Various factors which play important roles in this test need to be carefully controlled in order to standardize results from different laboratories. Alessi and Power (1971) noted that soil temperature had a much greater effect than seed depth on emergence of corn. Burris and Navratil (1979) felt that much of the cold test response was due to the temperature stress and little

to the soil. An interaction between low temperature and oxygen stress in cold wet soils adversely affected germination of garden beans (Ladron et al , 1980). Haskell (1949) found that genetic constitution, temperature, length of time in low temperature, and the nature of the germinating medium all significantly affected final germination of cold-tested corn. Substrate moisture, re-use of soil, time and place of soil collection, and method of storing the soil influenced the outcome of cold tests. However, the most difficult factor to control is the activity of the soil microflora (Svien and Isely, 1955). Seed injury, such as a break in the pericarp, can reduce the performance of a seed lot in the soil cold test (Crosier, 1958)

Widespread use of the cold test has been predominately restricted to corn, although procedures have been adapted for other crops such as pea (Clark and Baldauf, 1958), onion (Clark and Kline, 1962), and recently soybean. Effectiveness of seed treatments as well as germinability of the seed can be determined with this test (Clark, 1963). Field emergence of corn seed was more closely related to cold test performance than to seedling growth rate (Grabe, 1965). The cold test was very effective in evaluating corn seed deterioration during storage (Gill and Delouche, 1973). Variable results with the cold test have been obtained in soybean for predicting field performance (Johnson and Wax, 1978; Niles and Copeland, 1980, Polizotto, 1980).

Another physiological stress test that has recently been proposed is the accelerated aging test. This treatment requires storage of unimbibed seeds under adverse conditions of high temperature (41° C) and relative humidity (100%) for a short period of time. Germination capability decreases during storage with less vigorous seeds being the

most affected. A specific test procedure for accelerated aging has been recommended by McDonald and Phaneendranath (1978) for soybeans. The accelerated aging test was positively correlated to field emergence when used alone (Miles and Copeland, 1980, Polizotto, 1980) or in combination with other germination tests (TeKrony and Egli, 1977, Suryatmana et al., 1980). Recently, a methanol stress test has been proposed by Musgrave et al. (1980) that mimics the effects of accelerated aging.

Biochemical tests on the seed have been suggested to be associated with seed vigor. These tests monitor chemical reactions involved in cellular maintenance which are presumed to be related to the seeds' germinative capacity. Corn seed respiration rates (oxygen uptake) during the first 30 hours of germination were positively correlated with seedling growth 3 to 5 days after planting (Woodstock and Grebe, 1967). Measurements of respiration during the first 9 hours of germination were able to detect seed injury that affected subsequent seedling growth (Woodstock, 1965, Woodstock and Feeley, 1965). A specific respiration test was proposed by Woodstock (1966) for determination of seed vigor in corn. Gill and Delauche (1973) found that respiratory activity of corn seed decreased as storage time increased, resulting in greater seed deterioration. Respiration was also positively correlated with seed and seedling vigor in pea (Woodstock and Combs, 1967), wheat (Kittock and Law, 1968), and soybean (Burris et al., 1969).

Other parameters of respiration include ATP production and respiratory enzyme activity. Highly significant correlations between ATP and seed vigor were found in lettuce by Ching and Danielson (1972), in crimson clover, rape, and ryegrass seeds by Ching (1973), and in cauliflower by Lunn and Madsen (1981). However, Styer et al. (1980)

reported that seed ATP content did not correlate with reduced germination or vigor during storage. Glutamic acid decarboxylase activity (GADA) was positively correlated with corn seedling growth (Woodstock and Grabe, 1967) and was associated with longevity in storage (Gill and DeTouche, 1973, Grabe, 1965). Burris et al. (1969) demonstrated that GADA had little relationship to age of soybeans and Edje and Burris (1970) showed that GADA activity was not associated with either age or germination of soybean seeds. Scholl (1974) obtained a highly significant correlation between isocitratase activity and the percent of seedling fresh weight in the axis of cotton.

The tetrazolium (TZ) test relies upon the action of respiratory dehydrogenase enzymes with TZ to form a water-insoluble red pigment (McDonald, 1975). Important criteria to be considered are staining pattern and color intensity. This test provides an estimate of potential germination percentage, appraisal of embryo soundness, and diagnosis of causes of embryo disturbance (McDonald, 1975, Moore, 1966). However, results were not always correctly interpreted and adequate correlations with field emergence have not always been obtained (Miles and Copeland, 1980, Polizotte, 1980).

Measurements of electrical conductivity of seed leachates have been proposed as a vigor test (McDonald, 1975). The test is based on the assumption that weak seeds generally possess poor membrane structure which results in greater electrolyte loss and higher conductivity measurements. Less vigorous seeds were also observed to be frequently infected by fungi. This infection was considered a result of increased exudation of sugars and other metabolites (McDonald, 1975).

Weak seed lots of peas and French beans were detected by measuring the conductivity of seed leachates (Bradnock and Matthews, 1970, Matthews and Bradnock, 1967, 1968, Perry, 1970). More electrolytes were exuded from dead and low-vigor seed than from high-vigor seed in pea (Perry and Harrison, 1970). Yaklich and Abdul-Baki (1975) reported more sugars and amino acids were leached from deteriorated than from vigorous soybean seed. Recent positive correlations with field emergence using the conductivity method were obtained in corn (Joe et al., 1980, Tao, 1980), garden beans (Ladrer et al., 1980), navy beans (Suryatmana et al., 1980), peas (Mullett and Wilkinson, 1979), and soybeans (Miles and Copaland, 1980).

CHAPTER II
RELATIONSHIP OF THE ENVIRONMENT DURING SEED DEVELOPMENT WITH SEED VIGOR OF TWO ENDOSPERM MUTANTS OF CORN

Genetic mutations altering the type and quality of carbohydrates in corn endosperm have improved the quality of sweet corn. The mutant sugary (su) has long been incorporated into commercial sweet corn hybrids. Substitution of the shrunken-2 (sh2) gene for su increased total sugars fourfold at physiological maturity and decreased starch and water-soluble polysaccharides considerably (Laughnan, 1953). This sugar increase was due to reduced enzymatic activity of ADP-glucose pyrephosphorylase which is essential in the synthesis of starch (Dickinson and Preiss, 1969). Higher levels of sucrose and reducing sugars in sh2 were noted at all stages of development (Creech, 1965; Creech and McArdle, 1966). Cultivars of sh2 demonstrated superior post-harvest sugar retention (Garwood et al., 1976; Mann et al., 1971).

A major limiting factor to the acceptance and success of sh2 hybrids by the sweet corn industry has been low seed and seedling vigor. Hannah and Cantliffe (1977) observed that 9 days after planting, the percent stands of two sh2 hybrids were 38 and 66% compared to 82% for su. Germination and seedling vigor measurements indicated that sh2 wes significantly lower in both laboratory and field tests than su (Styer et al., 1980). The seeds of sh2 hybrids are smaller, lighter, and more easily damaged than su seeds due to the higher sugar and lower starch content of the endosperm. Dry weight of sh2 kernels was significantly

lower than su (Wann, 1980). The endosperm to embryo dry weight ratio was also lower for sh2 one primarily to a small endosperm. This combination of less starch and high sugar can increase susceptibility of the seed and seedling to fungal rots during germination in the field (Berger and Wolf, 1974, Pieczarka and Wolf, 1978).

The degree of maturation in corn as determined by various indicators can directly affect seed viability and vigor. Knittle and Burris (1976) reported that both shoot and root dry weight upon germination were highly dependent on date of harvest while germination percentages were high throughout development. Koehler et al (1934) determined that seedling vigor and field stands were much greater in the more mature developmental stages. Seedlings produced by immature seeds were much more susceptible to seedling diseases. In general, stands at low soil temperatures improved with an increase in seed maturity (Rush and Neel, 1954). Achievement of maximum kernel dry weight coincided with the appearance of a black closing layer in the placental region of corn kernels (Daynard and Duncan, 1969) and can be used as a visual indicator of physiological maturity (Daynard, 1972). Kernel moisture declined significantly during black layer development (Rench and Shaw, 1971). Delaying harvest past physiological maturity can reduce seed viability and vigor in soybeans caused by weathering and seed infection (Alexander and Hinson, 1973, TeKrony et al., 1980).

According to the Association of Official Seed Analysts' (AOSA) Seed Vigor Testing Committee, no one vigor test is likely to serve best for all crops (Woodstock, 1976). Due to the complexity of factors influencing seed vigor and wide variation of field conditions, a combination of methods may be preferable to a single test method. Vigor tests may

reproduce environmental stress expected in the field, such as the cold soil test (Grabe, 1965; Haskell, 1949) and the accelerated aging test (Delouche and Baskin, 1973, McDonald and Phaneendranath, 1978). In addition, vigor tests may involve other seed characteristics demonstrated to be correlated with field performance, such as seed size and weight (Ries and Everson, 1973, Seffer and Smith, 1974), seedling growth rate (Woodstock and Feeley, 1965), and leachate conductivity (Matthews and Bradnock, 1967). The results of the vigor test supplement the results of the germination test which are obtained under optimum laboratory conditions.

The cause of low seed vigor in the high-sugar genotype sh2 is not fully understood. It is not entirely known whether the low seed vigor is due to a smaller endosperm (Wann, 1980) or whether the embryo itself is genetically inferior and incapable of exhibiting strong vigor. The following experiments were designed to determine the relationship of production environment (field and greenhouse) during seed development with vigor of isogenic lines of sh2 and su sweet corn. Seeds of various maturities were physically characterized and evaluated for seed and seedling vigor under several test conditions.

Materials and Methods

Seed Production

Isogenic parental lines of corn homozygous for sugary (su) and shrunken-2 (sh2) were planted in the field at the Horticulture Unit at Gainesville in the spring of 1978 and 1979. F_1 seeds were obtained for analysis by cross-pollination by hand in 1978 and by natural means in

1979 of su ('Iobelle') and sh2 ('Florida Sweet'). The corn was grown according to Florida recommendations (Montelaro and Marvel, 1971).

The same genotypes were planted in 2-gallon pots containing soilless media in a greenhouse at various times of the year in 1978, 1979, and 1980. Seeds for analysis were obtained by cross-pollination by hand as noted above. All plants were adequately watered, fertilized, and sprayed with pesticides as needed. Temperature conditions were 30/19° ± 2° C (day/night).

The ears were harvested at different developmental stages (18 to 46 days post-pollination), husked, and dried for 2 weeks at 30° C, unless otherwise noted. The dried kernels were stored at 10° C and 45% relative humidity. Average weights of field- and greenhouse-grown sh2 and su seeds were determined on samples of 100 seeds.

Moisture Content

Ears of sh2 and su were harvested in the field in 1978 from 14 to 46 days post-pollination and husked. Samples from at least five ears per harvest date were cut from the cob, weighed, dried in an oven at 100° C for 24 hours, and re-weighed. Moisture contents were then calculated.

Optimum Temperature Vigor Test

Optimum germination and seedling vigor measurements were obtained by placing five replications of 10 seeds on moist nontoxic germination papers (Anchor Paper Co., St. Paul, MN). The papers were rolled up with a wax paper backing and placed in a germinator at 25° C. Germination counts were made daily for 7 days to determine rate and total percent

germination. Germination rate was calculated according to the formula of Shmueli and Goldberg (1971). Higher numbers indicate greater germination rates. After 7 days, the number of abnormal seedlings were noted, radicle and total seedling lengths were measured on germinated seedlings only, and fresh weights were recorded. Dry weights were determined by drying seedlings in an oven at 70° C for 48 hours. Fresh and dry weights of the germinated seedlings were expressed on an individual seedling basis.

Soil and Modified Cold Tests

To determine viability and vigor under stress conditions, five replications of 20 seeds per genotype and harvest date were subjected to a standardized cold soil test (Woodstock, 1976). Seeds were planted in plastic boxes (18 x 12.5 x 9 cm) containing predetermined amounts of field soil and water, sealed, and incubated at 10° C for 7 days, then transferred to 25° C for 4 days. Emergence rates were calculated as previously described. After 4 days, the soil was washed away, number of abnormal seedlings were noted, and radicle and total seedling lengths were measured on germinated seedlings only.

To compare viability and vigor under stress conditions, five replications of 20 seeds of field-grown sh2 and su of different developmental stages were placed on moist germination papers as noted above. The rolled papers were then incubated under identical conditions as the cold soil test. After 4 days, total percent germination and abnormal seedlings were determined, and radicle and total seedling lengths were measured on germinated seedlings only.

Accelerated Aging Test

At least 80 seeds in four replications of field-grown sh2 and su harvested 18 to 45 days post-pollination were placed on wire mesh screens in plastic containers (11 x 11 x 3.5 cm) above 40 ml water. The containers were sealed and incubated at 41° C for 4 days. Relative humidity in the containers was nearly 100%. After 4 days, moisture contents of sh2 and su seeds were 34 and 25%, respectively. The seeds were removed and dried at room temperature for 24 hours.

Optimum germination and vigor at 25° C were determined using the rolled paper method previously described. Two replications of 10 seeds per container were made. Total percent germination and abnormal seedlings, seedling lengths, and seedling dry weights were measured.

Two replications of 20 seeds per container were soaked in 100 ml deionized water for 24 hours at 20° C. After soaking, the leachate was filtered through a Buchner funnel and stored at 5° C. Electrical conductivity of the leachate was measured at room temperature using a conductivity meter and expressed as umhos/g of seed.

Statistical Analyses

All data were subjected to analysis of variance and significant differences between means determined by Duncan's new multiple range test. Statistics for data presented initially in figures in the text are included in tables in the Appendix.

Results

Kernels of sh2 had significantly higher moisture contents than su throughout development (Table 2-1). During the period 14 to 34 days

Table 2-1. Field moisture content of sh2 and su kernels harvested at various stages of development in 1978

Days post-pollination	Moisture content (%)	
	sh2	su
14	79[z]	77
18	76	70
22	74	66
26	72	58
30	66	53
34	61	48
38	50	45
42	--[y]	44
46	--	41

[z]All means significantly different within columns (Duncan's multiple range test) and rows (F-test) at the 5% level.

[y]No ears available

after pollination, su kernels lost 28% moisture compared to 18% for sh2. However, sh2 kernel moisture decreased more rapidly 34 to 38 days post-pollination. Ears of sh2 42 to 46 days after pollination were not available because heavy rains destroyed the remaining plants.

Greenhouse-grown seeds of either genotype generally weighed more than field-grown seeds throughout development (Figure 2-1, Table A-1). Seed weights of sh2 and su were significantly different, but increased at similar rates 18 to 26 days post-pollination. After this period, su seeds continued to increase in weight while sh2 seeds gained weight much more slowly.

The endosperm to embryo ratios of both su and sh2 are illustrated in Figure 2-2. Differences in the amount of endosperm are easily seen after the embryos have been stained with tetrazolium. Convolutions in the sh2 seeds are due to a lack of endosperm and to the high amounts of sugar and moisture retained during development which cause the endosperm to contract unevenly during drying.

Under optimum conditions, greenhouse-grown sh2 seeds germinated equally as well as both field- and greenhouse-grown su seeds 26 days post-pollination and later (Table 2-2). Germination percentages were variable in field-grown sh2 seeds, but remained consistently high in greenhouse-grown sh2 seeds after 26 days post-pollination. Field-grown su seeds 18 to 30 days after pollination produced significantly greater percentages of abnormal seedlings than the other three types. Germination rates did not always reflect differences in germination percentages, such as between field- and greenhouse-grown sh2 seeds 38 and 46 days post-pollination. Generally, radicle and seedling lengths were significantly greater for greenhouse-grown compared to field-grown

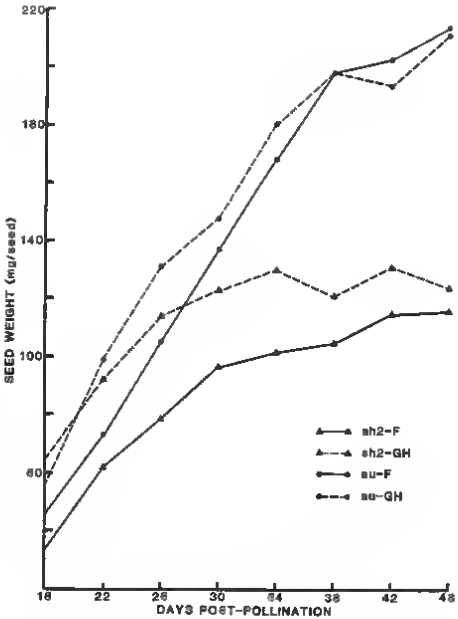


Figure 2-1. Weight of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30° C to 0% moisture

Figure 2-2. Longitudinal sections of mature sh2 (top) and su (bottom) seeds imbibed for 24 hours, cut, and stained in 0.1% tetrazolium chloride for 2 hours (The whole embryo is the red portion of the seed.)

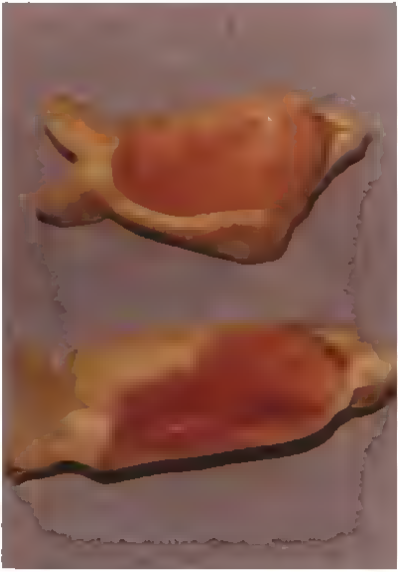


Table 2-2. Viability and vigor of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and germinated under optimum conditions

Type	Days post-pollination							
	18	22	26	30	34	38	42	46
				Germination (%)				
sh2 F	24b[z]	84b	24b	86a	80b	22b	62b	91a
sh2 GH	27b	70c	94a	95ab	97ab	96a	94a	93a
su F	94a	96a	17Ka	19Ka	17Ka	95a	94a	96a
su GH	96a	96a	92a	96ab	86ab	96a	95a	94a
				Abnormal seedlings (%)				
sh2 F	22b	18H	14bc	24b	24a	18H	15a	15a
sh2 GH	15b	13b	10c	19b	22a	20a	16a	14a
su F	54a	86a	46a	38a	26a	16a	25a	16H
su GH	8H	22b	28b	11b	23a	14a	15a	12a
				Germination rate index				
sh2 F	3 98c	4 32a	3 62b	4 32a	4.34a	3 94a	3 24b	4 49H
sh2 GH	3 89c	3 12b	4 40a	4 41a	4.47a	3 96a	3 86a	3 24b
su F	4 03b	4 54a	4 34a	4 24a	4.36a	4.22a	4 22a	4 86a
su GH	3 99a	4 94a	4 87a	4 65a	4 62a	4 52a	4 23a	4 22ab
				Radicle length (cm)				
sh2 F	9.2c	12.8b	12.6c	11 1b	10 6c	11 8b	8 8c	11 2c
sh2 GH	12 0H	13 6b	15 0H	12 1a	17 1a	12.8H	15.6ab	12 0H
su F	8.8c	12 4b	12.3c	12 0H	13 4b	13 1b	13 0H	15 0H
su GH	12 4a	18.2a	13 0H	18 6a	18 6a	18 1a	12 0H	18 1ab
				Seedling length (cm)				
sh2 F	15 9c	20 0H	19 0H	12.0H	19 1b	12.8b	13.0H	17 4c
sh2 GH	23 3b	20 0H	23 2a	24 6a	24 6a	18 0H	22 7b	25 6a
su F	13 2c	18.1c	18.1b	19 8b	12 4b	12 1b	17 8c	21 8b
su GH	28.4a	29 5a	25 4a	26 6a	29 0H	22.1a	26.3a	29 0a

[z]Mean separation in columns by Duncan's multiple range test, 5% level

seeds, regardless of genotype. Greenhouse-grown sh2 seeds generally produced longer radicles and whole seedlings than field-grown su seeds throughout development. Regardless of maturity, seedling fresh weights were significantly heavier for greenhouse-grown than field-grown sh2 and su seeds (Figure 2-3, Table A-2). Seedlings grown from greenhouse sh2 seeds had greater fresh weights than su seeds from the field 18 to 46 days post-pollination. Dry weights of su seedlings increased rapidly after 22 days post-pollination, while sh2 dry weights increased at a comparatively reduced rate (Figure 2-4). Greenhouse-grown sh2 seedling dry weights were significantly higher than field-grown sh2 throughout development, reflecting the differences noted in seedling fresh weights. Greenhouse-grown su dry weights remained fairly equal to field-grown su and did not equate with fresh weight differences.

A standard cold soil test was used to determine viability and vigor under stress conditions. Significantly greater emergence percentages were noted for greenhouse-grown than field-grown sh2 seeds after 38 days post-pollination (Table 2-3). Greenhouse-grown su seeds had higher emergence than field-grown su seeds 18 to 26 days after pollination. By 30 days post-pollination and after, field-grown su seeds had higher emergence percentages than their greenhouse counterparts. Mature greenhouse-grown sh2 seeds emerged equally as well as mature greenhouse-grown su. The percentage of abnormal seedlings was generally lower in the cold soil test compared to germination under optimum conditions. Greenhouse-grown su seeds 42 to 46 days old produced significantly greater percentages of abnormal seedlings than field-grown su seeds of the same age. Although emergence percentages and rates were related, there were no significant differences in emergence rates between

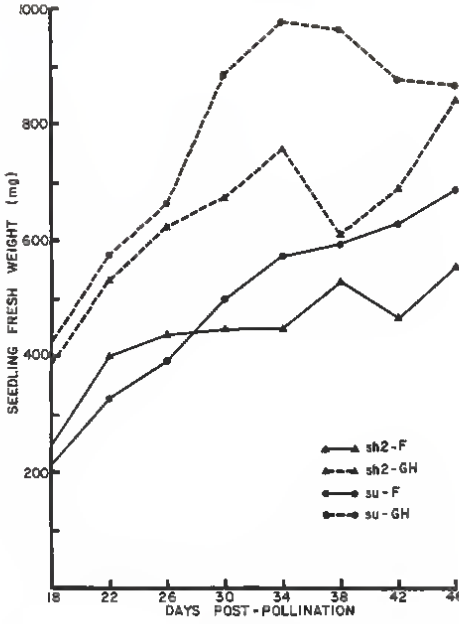


Figure 2-3. Fresh weight of seedlings of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and germinated under optimum conditions

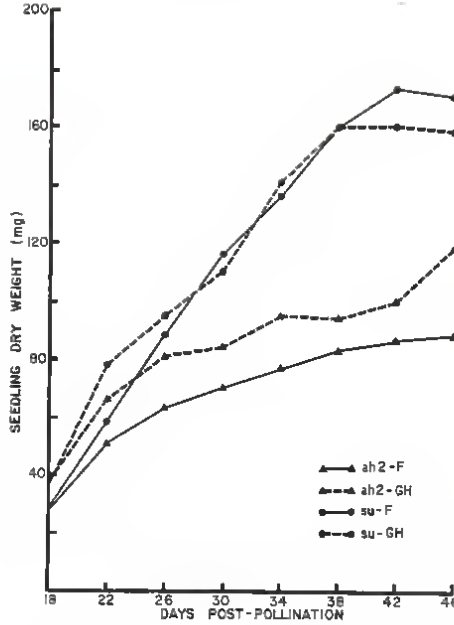


Figure 2-4. Dry weight of seedlings of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and germinated under optimum conditions

Table 2-3. Cold soil viability and vigor of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development

Type	Days post-pollination							
	18	22	26	30	34	38	42	46
				Emergence (%)				
sh2 F	4b[z]	14b	14c	4c	11a	16d	15c	40c
sh2 GH	2b	3b	6c	9c	1c	38c	62b	74b
su F	1b	16b	53b	77a	90a	86a	88a	90a
su GH	27a	47a	74a	69a	34b	68b	72b	72b
				Abnormal seedlings (%)				
sh2 F	3b	13a	7b	3b	9ab	6b	11b	14b
sh2 GH	0b	0b	0b	4b	1b	11ab	14b	12b
su F	0b	11a	21a	17a	5ab	11ab	14b	7b
su GH	15a	17a	18a	13a	13a	18a	36a	31a
				Emergence rate index				
sh2 F	07b	30b	21c	05b	18c	31b	23c	85c
sh2 GH	04b	05b	13c	13b	21c	01b	1.11b	1.64b
su F	71b	31b	96b	1.39a	1.96a	1.82a	1.70a	1.78ab
su GH	58a	1.18a	2.35d	1.27a	98b	1.01a	2.04a	1.93a
				Radicle length (cm)				
sh2 F	1.7ab	3.8c	6.0b	4.2c	2.6b	4.9c	3.2b	8.0b
sh2 GH	4.3ab	9.2b	6.0b	8.19c	8.1b	8.89c	8.8b	7.8b
su F	1.2b	4.9c	7.0b	8.4b	14.8a	10.5ab	11.1a	12.6a
su GH	4.5a	14.9a	15.3a	14.9a	12.1a	12.4a	11.4a	10.2ab
				Seedling length (cm)				
sh2 F	5.6bc	10.7b	11.2b	8.2c	8.8b	11.6b	2.9c	13.6b
sh2 GH	8.2ab	14.5b	4.4b	11.7bc	8.8b	13.8b	13.7b	16.6ab
su F	2.1c	4.0b	14.1b	17.0b	17.3a	17.1ab	16.4ab	22.3a
su GH	11.8a	27.1a	27.5a	28.7a	19.7a	21.1a	20.4a	21.5a

[z]Mean separation in columns by Duncan's multiple range test, 4% level

field- and greenhouse-grown su seeds 38 to 46 days after pollination. In sh2 seeds 38 to 46 days old from the field and greenhouse, no significant differences were observed in radicle and seedling lengths, except for the 42-day-seedling lengths. Radicle and seedling lengths were significantly longer for greenhouse-grown su compared to field-grown su seeds 18 to 30 days post-pollination, but were not significantly different at later maturity dates. Generally, radicle and seedling lengths were greater for su than sh2 throughout development, except that fully mature greenhouse-grown su and sh2 radicle and seedling lengths, as well as emergence percentages, were not significantly different.

Another, less severe, stress test that was used to classify vigor was the modified cold test. When compared to the cold soil test, germination percentages were much higher for sh2 seeds in the modified cold test at all stages of development (Figure 2-5, Table A-3). Germination of su seeds 22 days of age and older exceeded the 90% level in the modified cold test. Generally, there were more abnormal seedlings produced from mature than immature sh2 seeds while mature su seeds produced less abnormal seedlings than immature su seeds (Figure 2-6). Radicle lengths from sh2 seeds in the cold soil test were less than from sh2 seeds in the modified cold test throughout development, although this difference was not always significant (Figure 2-7). Shorter radicles were noted for su until 30 days post-pollination in the modified compared to the cold soil test. Differences in seedling lengths between sh2 in modified and soil cold tests were generally not apparent, but were greater between su in the modified and soil cold tests (Figure 2-8). Seedling lengths of su increased more rapidly in the soil than modified tests. Whole seedling measurements of su 34 to 46 days post-pollination,

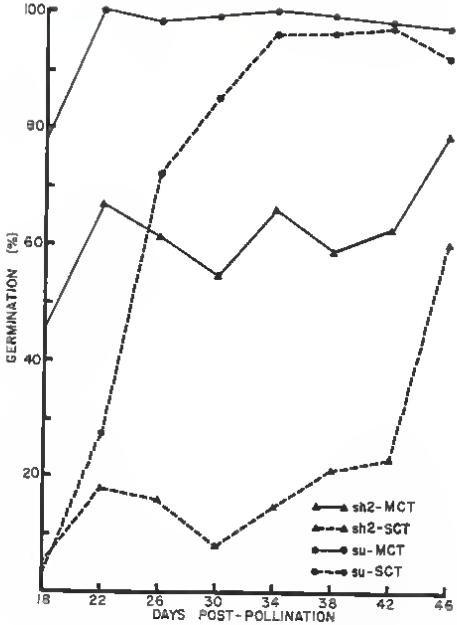


Figure 2-5 Germination of field-grown sh2 and su seeds harvested at various stages of development as determined by a modified (MCT) and soil cold test (SCT)

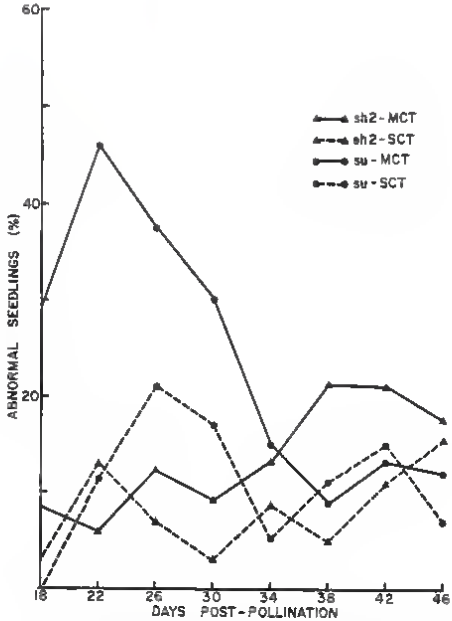


Figure 2-6. Percentage of abnormal seedlings of field-grown sh2 and su seeds harvested at various stages of development as determined by a modified (MCT) and soil cold test (SCT)

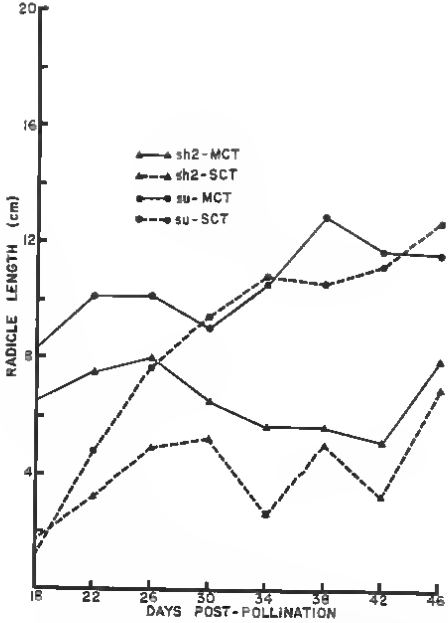


Figure 2-7 Length of seedling radicles of field-grown sh2 and su seeds harvested at various stages of development as determined by a modified (MCT) and soil cold test (SCT)

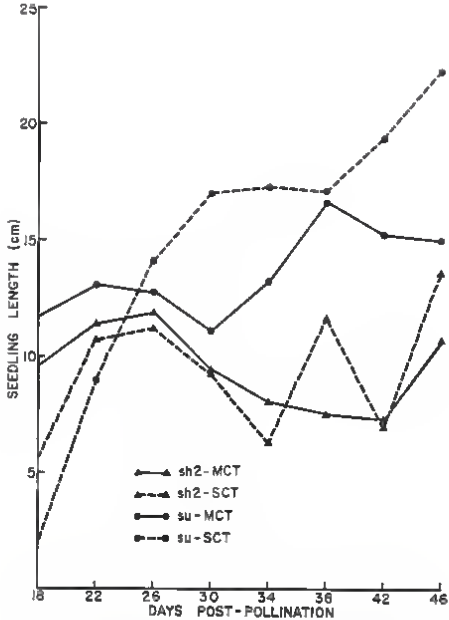


Figure 2-8 Length of whole seedlings of field-grown sh2 and su seeds harvested at various stages of development as determined by a modified (MCT) and soil cold test (SCT)

excluding 38 days, indicated significant differences between the modified and soil cold tests while germination percentages did not.

Germination percenteges of sh2 seeds throughout development were always significantly lower after accelerated aging then before this stress test (Figure 2-9, Table A-4). Interference by Aspergillus flevus, a storage fungus, particularly influenced germination of 34-day-old sh2 seeds after accelerated aging. At 22 and 26 days post-pollination, germination percentages of aged su seeds equeled these of nonaged su seeds, but declined rapidly in seeds older then 26 days. Significant differences in germination were observed after mature seeds of either genotype were aged. Seedling lengths of aged sh2 seeds were significantly less than nonaged sh2 seeds after 18 days post-pollination (Figure 2-10). After accelerated aging, seedling lengths of su seeds 22 days and elder were significantly longer than before aging. Seedling dry weights of sh2 and su were not significantly different until seeds were older than 22 days (Figure 2-11). After this period, su seedlings continued to rapidly increase in dry weight while sh2 seedlings gained weight more slowly. Accelerated aging of both sh2 and su seeds had little effect throughout development on dry weights of the resultant seedlings.

Shrunken-2 seeds elder than 22 days had significantly more electrolyte leakage than similarly aged sh counterparts (Figure 2-12). In su seeds, the amount of leakage declined rapidly from seeds 18 to 26 days post-pollination, then leveled off thereafter. Conductivity measurements were significantly higher for aged than nonaged sh2 seeds throughout development, except at 18 and 38 days post-pollination. No significant

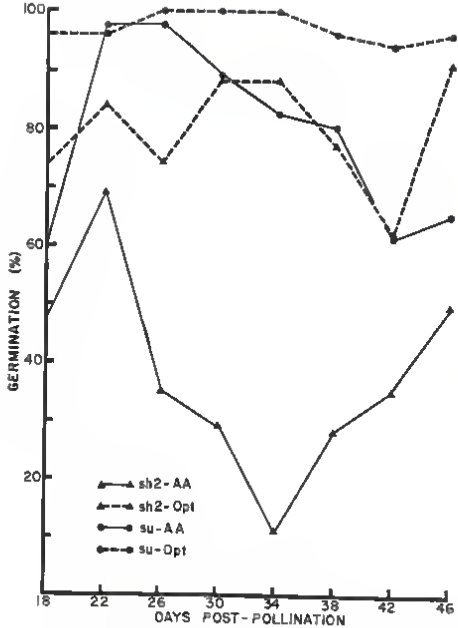


Figure 2-9. Germination of field-grown sh2 and su seeds harvested at various stages of development as determined under optimum (Opt) and accelerated aging (AA) conditions

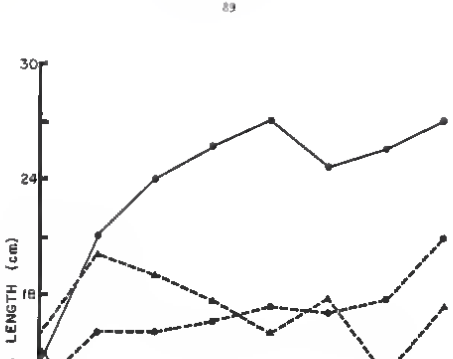


Figure 2-10. Length of seedlings of field-grown sh2 and su seeds harvested at various stages of development as determined under optimum (Opt) and accelerated aging (AA) conditions

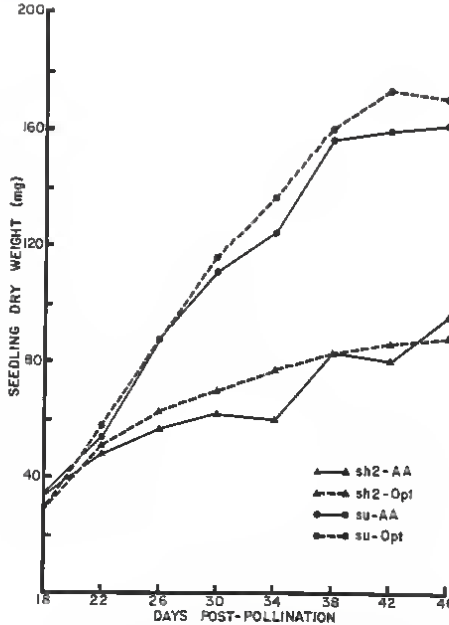


Figure 2-11. Dry weight of seedlings of field-grown sh2 and su seeds harvested at various stages of development as determined under optimum (Opt) and accelerated aging (AA) conditions

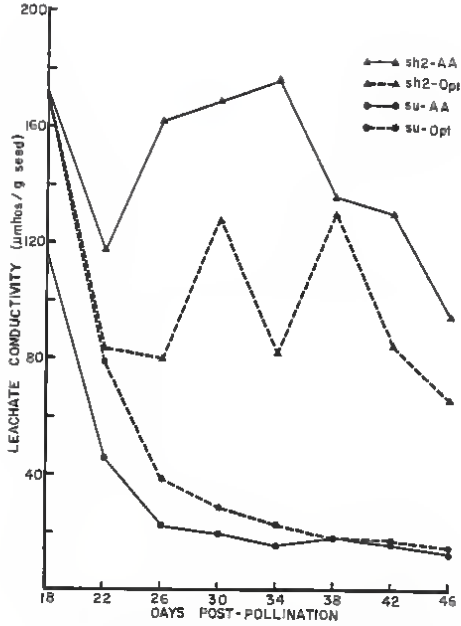


Figure 2-12 Conductivity of leachate of field-grown sh2 and su seeds harvested at various stages of development as determined under optimum (Opt) and accelerated aging (AA) conditions

differences in leakage were noted between aged and nonaged sh seeds 25 days and older.

Discussion

The higher moisture content of sh2 kernels throughout development can lead to problems in the field. Pathogens may be able to infect ears and kernels during a longer developmental period. Their entrance is facilitated by insects (Smeltzer, 1959) which also may feed on the kernels for a greater length of time due to the higher kernel moisture and anger retention (Creech, 1965). Infection may be aided by moisture in the silks, kernels, and cob (Koehler, 1942, Warren, 1978). Rainfall in the spring growing season in Florida can lead to higher moisture levels during seed maturation, thus increasing the potential incidence of ear, kernel, and stalk rots (Shurtleff, 1977).

One factor regulating field moisture loss in corn is the development of a closing or black layer in the placental region of the kernel (Kiesselbach and Walker, 1952). This layer, when complete, effectively separates the mature kernel from the rest of the plant. Movement of nutrients and moisture across the placental region slows down with maturity and is then cut off by the formation of the black layer. Thus, the filling stage of seed development is completed and further moisture loss must occur through the pericarp. The black layer can be associated with the attainment of maximum kernel dry weight (Daynard and Duncan, 1969) and physiological maturity (Daynard, 1972). A significant kernel moisture loss during black layer development was demonstrated by Kench and Shaw (1971). Although no observations were made in the present study as to when this layer developed in sh2 and su kernels,

differences in moisture loss may possibly be due to variations in time of initiation of black layer development.

Moisture loss by the kernels can also be controlled by the pericarp (Purdy and Crane, 1967) and by hydrophilic compounds in the endosperm, such as sugars (Ness and Crane, 1970b). Therefore, sh2 kernels may not be losing moisture as rapidly as su kernels because of the high sugar concentration of sh2 throughout development (Creech, 1965, Creech and McArdle, 1966). After harvest, ears of sh2 and su were dried at 30° C for two weeks to reach a stabilized seed moisture content of approximately 8%. Since sh2 and su kernels had significantly different moisture contents at the same field developmental stage, their rates of moisture loss during artificial drying would also be expected to be different. Personal observations indicated that the rates were not equal, with su kernels achieving the desired low moisture level faster than sh2 kernels. Upon drying, kernels of sh2 shriveled far more than su kernels, regardless of developmental stage. The lack of endosperm starch, together with high amounts of sugars, was primarily responsible for the shrunken appearance of these seeds (Laughnan, 1953).

At maturity, su seeds weighed twice as much as sh2 seeds. The differences in seed weights became more evident after 22 days post-pollination. Starch accumulation (Chapter III) continued in su, but not in sh2, seeds after this time, resulting in a greater amount of endosperm for su. The endosperm to embryo dry weight ratios have been calculated to be approximately 4.3 and 2.6 for mature su and sh2, respectively (Styer and Cantliffe, 1981, Mann, 1980). This ratio may reflect the relative amounts of nutrient reserves available to the embryo during germination.

Greenhouse-grown seeds, particularly sh2, weighed more than field-grown seeds at the same stage of development. The climate in the greenhouse was very favorable for maximum plant growth and seed development. No competition existed between plants for water, nutrients, or sunlight, and pest damage was minimized. These factors, which comprise the greenhouse environment, optimized seed development, resulting in the production of heavier and more vigorous seeds. Physical characteristics, such as size and weight, of some seeds have been used as an estimation of vigor (Ries, 1971, Ries and Everson, 1973, Scaife and Jones, 1970, Smith et al., 1973).

The stage of development at which all seeds were harvested did not appear to greatly affect germination under optimum conditions. Field- and greenhouse-grown 18-day-old su seeds that had developed to only one-fourth to one-third their potential seed weights germinated as well as fully mature seeds. Greenhouse-produced sh2 seeds older than 22 days had nearly reached their final seed weights and germinated above 90%. However, seedling growth measurements demonstrated developmental differences in all seed types that were not related to germination percentages. These results agree with those of Knittle and Burris (1976), who found that both corn shoot and root dry weights were highly dependent on date of harvest, even though germination percentages were high over all harvest dates (35 to 98 days after silking). They also noted that dates of maximum vigor and kernel maturity patterns were specific for each hybrid tested. Fussell and Pearson (1980) determined that seed viability and vigor of pearl millet were unaffected by the termination of grain development at or after mid-grain fill. Before this period, viability and vigor were low and seeds were more susceptible to

disease at germination. With immature seeds of sh2 and su, poor seedling development under optimum conditions may be related to low endosperm reserves. However, the erratic performance of increasingly mature field-grown sh2 seeds appeared to be due to interference by fungi on or in the seeds, producing seed and seedling rot (personal observation).

Although maturity had only a minor influence on germination of sh2 and su seeds under optimum conditions, such was not the case when seeds were germinated under stress conditions. The cold soil test placed the greatest stress on seed germination among the various tests used. Under these conditions, only the most vigorous seeds germinated and grew. Seeds that might have produced abnormal seedlings under optimum conditions did not germinate under the severe stress of the cold soil test. Immature seeds of both genotypes germinated very poorly, possibly due to their small size and susceptibility to pathogens during germination.

Viability of sh2 seeds in the cold soil test was generally much lower than su. Field-grown su seeds emerged above 70% only when 30 days or older. This large difference in developmental time between sh2 and sn to attain high viability and vigor may be due to genotypic differences in physiological development and maturation. The amount of endosperm reserves may not be as adequate, for example, in 30-day-old sh2 as in su. If these reserves are not sufficient, then viability and vigor, particularly under stress conditions, will be adversely affected. Another reason for developmental differences in viability and vigor between genotypes may be seed leakage. Seeds of sh2 leached large amounts of nutrients when imbibed until fully mature. Seed exudates, primarily carbohydrates and electrolytes, have been shown to stimulate pathogens in the soil, which may then attack and kill or injure the

germinating seed (Schroth and Cook, 1964, Short and Lacy, 1974). Cold soil conditions increase seed exudation while delaying germination, thus allowing pathogens to overcome the seed (Schroth et al., 1966, Short and Lacy, 1976). If pathogens are located inside the seed, leakage may have a more profound effect. Immature su seeds may behave similarly to sh2 seeds. However, leakage decreased more rapidly in sn with increasing maturity and helped to account for the high viability and vigor earlier in development. The erratic emergence and seedling growth of greenhouse-grown sn seeds throughout development cannot be adequately explained.

Determination of maturity in sh2 poses particular problems not associated with su. The usual methods of determination, such as kernel moisture content, kernel dry weight, and germination percentages under optimum conditions, do not hold true for sh2 due to reasons previously stated. If these seeds are harvested early because of a short growing season or frost, their subsequent performance in the field may not be as good as expected.

Accurate determinations of viability and vigor can be made through the use of stress tests. In the present study, these tests included modified cold, cold soil, and accelerated aging. Although the modified cold test was easier to conduct, the lesser degree of severity of stress this test placed on germinating seeds did not allow adequate detection of viability and vigor differences as noted in the cold soil test. For example, germination of sn seeds 18 to 30 days post-pollination was very high in the modified cold test, with only 18-day-old seed showing lower viability. However, under cold soil conditions, large differences in germination were observed throughout this developmental period.

The accelarated aging test, which has been standardized and used more in recent years (McDonald and Phaneendranath, 1978), presented some problems and possibilities. Some variations in viability and vigor measurements after aging of sh2 seeds 30 to 46 days post-pollination were due to interference by storage fungi, a major drawback of this test. Surface sterilization of seeds before aging may alleviate the fungal problem, but should be investigated for any interaction with seed germination and seedling growth. One noticeable effect of accelerated aging on su seeds was an increase in seedling lengths. This increase may be the result of the seeds becoming 'primed' through the aging process, which would give them a boost when germinated. Of the various measurements conducted, only leachate conductivity appeared to show promise as a vigor indicator. Differences were observed in leakage of sh2 seeds before and after accelerated aging. Conductivity has been shown to be highly correlated with vigor and field performance (Matthews and Bradnock, 1967).

The production environment can definitely effect viability and vigor by interacting with seed development and maturation. In the greenhouse, the environment was much more favorable than in the field. Optimum climate, lack of interplant competition, and exclusion of pathogens and insects all combined to produce healthier plants and heavier, noninfected seeds. Generally, greenhouse-grown sh2 seeds had higher viability and vigor than field-grown seeds throughout most of development. Under severe stress conditions during germination, only mature (38 to 46 days post-pollination) sh2 seeds from the greenhouse exhibited significantly greater emergence than their counterparts from the field. Thus, vigor of sh2 seeds can be increased by production in a

favorable environment. Greater endosperm reserves and reduced pathogenic activity may lead to an increase in vigor. This does not appear to be the case with su. Seeds of this genotype can withstand more severe environmental conditions during seed development, while still producing relatively high-quality seeds.

Seed development and vigor may be influenced by the temperature of the production environment. Fussell and Pearson (1980) determined that the temperature at which pearl millet grains had developed did not affect seed viability. However, grains that had developed at 21/16° C (day/night) produced seedlings of greater height and dry weight than those from grains which had developed at higher temperatures. This difference in vigor was consistent with the greater carbohydrate and protein reserves of the grains produced at low temperatures. The high field temperatures associated with kernel development during the spring in Florida may be limiting, to some extent, endosperm reserve accumulation in sh2 kernels. This limitation would result in smaller and less vigorous seeds than normal.

Corn seeds produced in short growing seasons run the risk of suffering damage due to frost or freeze. Major (1980) simulated killing frosts by spraying corn plants with paraquat. Generally, development was affected less than yield, with kernels continuing to fill even after leaves were killed. The earlier the treatment was imposed, the greater was the reduction in growth rate of grain. Reduction of corn seed viability due to freezing in the field depended on the low temperature reached, duration of exposure, seed moisture content, variety, husk protection, physiological maturity of seed, and rate of drying after freezing (Rossman, 1948). The high kernel moisture during development

and slow attainment of physiological maturity of sh2 seeds may make this genotype more vulnerable to frosts and freezes than su, thereby further reducing seed viability and vigor in northern production regions.

Production environment and seed development may influence additional factors, such as structure, composition, and leakage of seeds, and pathogenic infection. These factors, in turn, can affect seed viability and, possibly, vigor. The extent of their influence can vary with the seed type. Further work is reported in subsequent chapters on the effects that these factors have on viability and vigor of developing sh2 and su seeds.

Summary

Seeds of two corn endosperm mutants, sh2 and su, were produced in the field and greenhouse and harvested 18 to 46 days post-pollination. Moisture contents of field-grown kernels were determined immediately after harvest. All ears were dried at 30° C for 2 weeks to 8% moisture. Seeds were weighed and tested for viability and vigor under a wide range of conditions. The tests included (1) optimum temperature (25° C), (2) modified cold, (3) cold soil, (4) accelerated aging, and (5) leachate conductivity. Measurements were made of germination and seedling growth.

Kernel moisture of sh2 was significantly greater than su throughout development. Seeds of su continued to accumulate dry weight rapidly after 26 days post-pollination, while sh2 seeds had nearly attained their final weight by this age, resulting in a greater amount of endosperm for su. Greenhouse-grown sh2 seeds weighed more than field-grown sh2 18 to 46 days post-pollination.

Germination and seedling growth of greenhouse-grown sh2 seeds under optimum conditions were greater than field-grown sh2 when seeds were older than 26 days. Greenhouse-grown sh2 seeds 26 days and older germinated equally as well as field- and greenhouse-grown su and seedling growth was comparable. Under cold soil test conditions, greenhouse-grown sh2 seeds had significantly greater emergence than field-grown sh2 only when 38 days and older. Mature greenhouse-grown sh2 and su seeds were equal in viability and vigor in the cold soil test.

The modified cold and accelerated aging tests were conducted on field-grown seeds only. Compared to the cold soil test, germination of sh2 seeds in the modified cold test was much higher throughout development. Viability and vigor differences between immature seeds of both genotypes were not distinguishable in the modified cold test, but were very noticeable in the cold soil test. The lack of soil pathogenic stress in the modified cold test may be responsible. In the accelerated aging test, interference by Aspergillus flavus, a storage fungus, was a major problem, particularly with sh2 seeds 30 days post-pollination and older. Accelerated aging of su seeds resulted in a beneficial 'priming' effect on seedling lengths. Seedling dry weights of either genotype were not affected by aging. Leechate conductivity indicated vigor differences of sh2 and su seeds during development, with differences increasing after aging.

From the results of this study, the stressful field environment in Florida may reduce seed size and vigor of sh2 during development. Viability and vigor of sh2 was increased by producing seeds in the more sheltered greenhouse environment. Greater endosperm reserves and reduced pathogenic activity on the seeds may have led to increased vigor

of greenhouse-grown sh2 seeds Sugary seeds were not affected to the same extent as sh2 by production environment Seed development of sh2 appeared to be slower than su, leaving this genotype more susceptible to damage by pests and the environment.

CHAPTER III
CHANGES IN SEED STRUCTURE AND COMPOSITION DURING DEVELOPMENT AND THEIR EFFECTS ON LEAKAGE AND SEED VIGOR OF TWO ENDOSPERM MUTANTS OF SWEET CORN

Introduction of the mutant gene shrunken-2 (sh2) in place of the standard sugary (su) gene in sweet corn increased total sugar content, mostly sucrose, two to three times at edible maturity (Creech, 1965, Wann et el., 1971). Starch levels in sh2 were reduced up to 38% compared to su 16 to 28 days post-pollination (Creech, 1965). At physiological seed maturity, sh2 contained four times more total sugars than su (Laughman, 1953) and were less vigorous than su under laboratory and field conditions (Styer et el., 1980). Poor seed vigor of sh2 has been related to small endosperms (Wann, 1980), but the higher sugar content of the kernel during seed development has also been associated with an increase in rot by pathogens during germination (Berger and Wolf, 1974, Pieczarka and Wolf, 1976). Leakage of sugars from sh2 kernels may be influencing seed rot.

Seed lots that leak heavily are most prone to pre-emergence mortality. Permeable corn seeds, as indicated by a high concentration of soluble materials leaching during a soaking period, were more susceptible to emergence failure under cold soil conditions (Tatum, 1954). At less than optimum soil temperatures, germinating seeds and seedlings had slower growth and more seed exudation, leaving them highly susceptible to attack by microorganisms (Schroth et el., 1966). Conditions which favored high amounts of nutrient exudation generally corresponded with

conditions which favored high levels of pathogen spore germination around seeds, leading to seedling decay (Short and Lacy, 1976).

Imbibition temperature and seed moisture content influence water uptake rate and leakage. Soybean seeds were increasingly sensitive to low temperature during imbibition, with leakage the greatest at temperatures below 15° C (Bramlage et al., 1978, Leopold, 1980). Seeds at low moisture content (5 to 8%) suffered imbibitional chilling injury, whereas seeds at 13 to 20% initial moisture exhibited no symptoms of injury during germination (Simon, 1974). Leakage was very low if pea embryos already had water contents of 30% or more (Simon and Wiebe, 1975). Tully et al. (1981) determined that sensitivity to imbibitional chilling was a consequence of the rate of cold water uptake.

The seed coat and related protective structures restrict or regulate water uptake and solute leakage by acting as a barrier to water diffusion. In wheat, the seed coat or testa is the layer offering the greatest resistance to water entry (Hinton, 1955). Lima bean seed coats allow the seed to avoid injury at low temperature and moisture stress (Pollock and Toole, 1966). Cracking or removal of the seed coat increased the velocity of water uptake and solute leakage. Seed coat cracking and leakage was greater in white-seeded than black-seeded bean cultivars before emergence, making them more susceptible to attack by soil pathogens (Presed and Weigle, 1976). The degree of lignification and thickness effect the permeability of the seed coat. Lignin comprised about 15% of the total seed coat weight of colored lima bean seeds, but only about 1% of white seeds (Kannenburg and Allard, 1964). Colored snap bean seeds had greater seed coat dry weight and thickness and less permeability to water than white seeds (Wyatt, 1977).

The following experiments were designed to determine the effects of seed structure and composition on leakage and seed vigor of *sh2* and *su*, and how the environment during seed development influenced these seed quality factors.

Materials and Methods

Seeds of *sh2* and *su* corn were produced in the field and greenhouse and harvested at various developmental stages as previously described (Chapter II)

Respiration

Respiration rates of imbibing seeds were measured manometrically in a Gilson respirometer. Mature 46-day-old field-grown *sh2* and *su* seeds were imbibed in distilled water at 25° C for up to 96 hours. At specific intervals, four replications of five seeds of each type were placed in flasks with 1 ml of distilled water. Ten percent potassium hydroxide (w/v) and a paper wick were placed in the flask center well as a carbon dioxide absorbent. After 30 minutes of equilibration at 25° C, oxygen uptake in µl/hr/kernel was determined.

Imbibition

Two replications of 20 seeds of mature 46-day-old field- and greenhouse-grown *sh2* and *su* were placed in petri dishes with filter paper (Whatman No. 3) and allowed to imbibe distilled water at 25° C. The increase in fresh weight after blotting of the seeds was measured after 1 to 60 hours of imbibition.

Leachate Analysis

Four replications of 25 seeds of field- and greenhouse-grown sh2 and su 18 to 46 days post-pollination were soaked in 100 ml deionized water for 24 hours at 20° C. After soaking, the leachate was filtered through a Buchner funnel and stored at 5° C. Electrical conductivity of the leachate was measured at room temperature using a conductivity meter and expressed as umhos/g of seed. Amount of potassium in the leachate was determined with a Beckman DU flame spectrophotometer (Chapman and Pratt, 1961) and calculated in ppm/g of seed.

Total carbohydrates were obtained using the anthrone-sulfuric acid method (Umbreit et al., 1959). One milliliter of sample was diluted with 2 ml of water and added to a test tube containing a 6 ml solution of 2 g anthrone dissolved in 1 liter of concentrated sulfuric acid. After quickly mixing and cooling in an ice bath, the tubes were transferred to a boiling water bath for 10 minutes and then cooled immediately. Samples were measured in a spectrophotometer at 620 nm with a glucose standard curve ranging from 12 to 120 µg/ml.

Sugars were measured by gas-liquid chromatography (GLC) as described by Ferguson et al. (1979). This method was compared to a modified Somogi method (Crenin and Smith, 1979) and found to be more accurate. Four milliliters of leachate were oven-dried in a vial at 70° C. Hexane was added to solubilize lipids and sugars were redissolved with distilled water. After mixing thoroughly, the hexane-lipid phase was removed and the vials oven-dried at 70° C. No sugars were lost in the hexane phase.

Oximes of the dried sugars were made by addition of 0.1 ml of STOX Oxime-Internal Standard Reagent (Pierce Chemical Co., Rockford, IL)

which contained 25 mg hydroxylamine hydrochloride/ml pyridine and 8 mg phenyl-β-D-glucopyranoside/ml pyridine as an internal standard. The vials were incubated for 20 minutes at 70° C and cooled to room temperature before silylation.

The oxime-sugars were silylated by the addition of 0.1 ml of N-trimethyl silylimidazole followed by vortexing the sample for 30 seconds. The vials were incubated for 30 minutes at room temperature before injection. One microliter of the silylated sugars was injected into a Hewlett Packard 5710A gas chromatograph programmed at 150° C for 2 minutes followed by a linear rise in temperature to 250° C at a rate of 8° C/minute. Injection port temperature was 300° C and detector temperature was 350° C. The column was stainless steel (8' x 1/8") packed with 3% OV-17 on Chromosorb W(HP) 80/100 mesh (Alltech Associates, Inc., Deerfield, IL). The carrier gas was helium at 40 ml/minute.

Sugars measured were fructose, glucose, and sucrose, and expressed as mg/g seed. Total sugars were obtained by totaling the amounts of fructose, glucose, and sucrose. Four replications were made per seed type and harvest date.

Carbohydrate Analysis of Dry Seed

Total carbohydrates were determined by mixing 50 mg finely ground dry seed with 20 ml of 0.5 N sodium hydroxide in a large test tube and placing in a boiling water bath for 30 minutes. The tubes were then cooled, neutralized with 20 ml of 0.5 N acetic acid, mixed thoroughly, and filtered through a Buchner funnel (Dekker and Richards, 1971). One milliliter of each filtrate was diluted with 2 ml water and analyzed by the anthrone-sulfuric acid method as previously described. Four replications were conducted of each seed type and harvest date.

Sugars were extracted by mixing 500 mg of finely ground dry seed with 25 ml of 95% ethanol in a large test tube and placing in a water bath at 80° C for 60 minutes. The tubes were then cooled and filtered through a Buchner funnel. The ethanol filtrate was adjusted to 50 ml volume and sugars were determined by the GLC method previously described. Four replications of each seed type and harvest date were performed. The residue from the ethanol extraction was dried in an oven at 70° C for 24 hours, weighed, and saved for starch determination.

Starches were extracted as previously described for total carbohydrates (Dekker and Richards, 1971), except 100 mg of residue were mixed with 15 ml of 0.5 N sodium hydroxide and neutralized with 15 ml of 0.5 N acetic acid. A 2 ml aliquot of each extract was incubated with 2 ml of an amyloglucosidase solution (10 mg amyloglucosidase from Rhizopus mold, Sigma Chemical Co., dissolved in 1 ml of 0.1 M sodium acetate buffer, pH 4.5) in a water bath at 55° C for 60 minutes. After cooling to room temperature, a 25 µl aliquot was injected into a YSI Model 27 Industrial Analyzer (Yellow Springs Instrument Co., Yellow Springs, OH) which measures glucose by an oxidase enzyme membrane technique. Amounts of glucose were then converted to mg starch/g dry weight seed. Four replications were conducted of each seed type and harvest date.

Freeze-Dried Ears

Kernels from freeze-dried ears of field-grown sh2 and sn harvested at various developmental stages in 1979 were ground to a powder, extracted with ethanol, and sugars and starch determined as previously described. Sample dates for sh2 were 15, 22, and 38 days, whereas su

included 18, 22, and 46 days post-pollination. The most mature stage available of sh2 and su freeze-dried ears was 38 and 46 days post-pollination, respectively.

Scanning Electron Microscopy

Seeds for scanning electron microscope observations were freeze-dried and mounted on aluminum stubs with Tube Coat (G.C. Electronics Co., Rockford, IL). The samples were oven-dried at 70° C overnight and sputter-coated in a Technics Hummer V specimen coater with 60 nm of gold-palladium. Specimens were viewed in a Hitachi scanning electron microscope (SEM), Model S-450, using an accelereted voltage of 20 KV.

Light Microscopy

Seeds were cut longitudinally with a single edge razor blade and exposed to osmium tetroxide vapors for at least 1 hour. The halves were fixed in 2.5% gluteraldehyde (v/v), 1% acrolein (v/v), and 5% sucrose (w/v) in 0.05 M sodium cacodylate buffer, pH 7.5, overnight at room temperature. At least two changes of the fixative were made during this time. Samples were washed four times in buffer for 30 minutes each and post-fixed overnight at 4° C in 0.05 M sodium cacodylate-buffered 1% osmium tetroxide. The seed halves were then washed four times for 30 minutes in distilled water and stained en bloc overnight in 0.5% aqueous uranyl acetate (w/v). After washing once in distilled water, samples were dehydrated in acidified 2,2-dimethoxypropane (DMP) three times for 30 minutes, then placed in absolute acetone overnight (Poster and Tucker, 1976). The pieces were infiltrated with and embedded in a modified Spurr's resin (Ringo et al., 1978) containing 2.45 g nonenyl succinic

enhydride (NSA), 0.52 g vinyl cyclohexene dioxide (VCD), 0.48 g Quetol 651, and 0.045 g dimethylaminoethanol (DMAE). Sections 1 to 2 µm thick were cut with a glass knife on a Sorvall MT-5000 ultramicrotome and stained for light microscopy with 135 mg basic fuchsin and 365 mg toluidine blue O in 100 ml of 25% ethanol.

Statistical Analyses

All data were subjected to analysis of variance and significant differences between means determined by Duncan's new multiple range test. Statistics for data presented initially in figures in the text are included in tables in the Appendix.

Results

Imbibition rates for the first 60 hours of germination were significantly greater for mature sh2 than su seeds (Figure 3-1, Table A-5). Field-grown sh2 seeds imbibed significantly more water than greenhouse-grown sh2 from 1 to 24 hours, but significantly less water after 24 hours. Mature su seeds produced in the field and greenhouse had equal imbibition rates until 36 hours, at which point greenhouse-grown su seeds imbibed significantly greater amounts of water than field-grown su. Radicle emergence of all seed types generally occurred after 36 hours of imbibition.

Measurements of electrical conductivity of leachates indicated that immature seeds (up to 26 days post-pollination) of sh2 and su leaked more than older seeds (Figure 3-2, Table A-6). These levels continually declined with maturity, except in field-grown sh2, which had unusually high leakage at 30 and 36 days post-pollination. Field-grown sh2 seeds

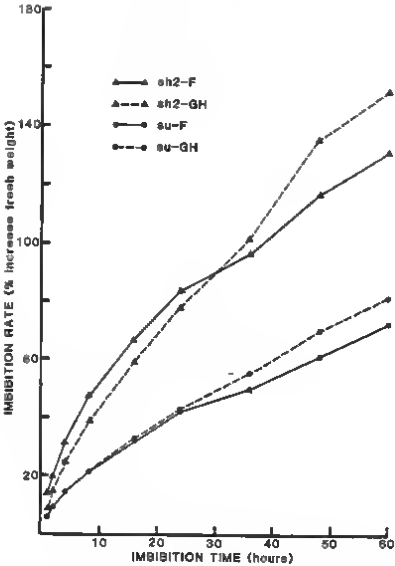


Figure 3-1. Time course of imbibition of mature (46-day-old) field-grown (F) and greenhouse-grown (GH) sh2 and su seeds

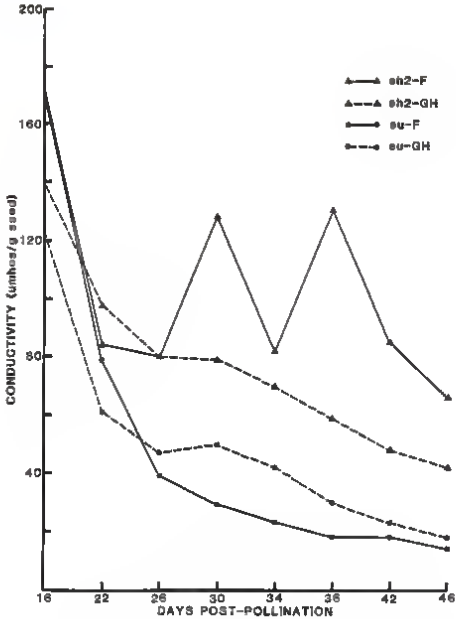


Figure 3-2. Electrical conductivity of leachate of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development

consistantly leaked more electrolytes than greenhouse-grown sh2 38 days post-pollination and later. Leakage of greenhouse-grown sh2 seeds followed a similar pattern as su during development, but continually leaked more than su when 22 days or older. Greenhouse-grown su seeds were significantly leakier than field-grown su 30 and 34 days post-pollination but not thereafter. At maturity, leachate conductivity levels were equal for field- and greenhouse-grown su seeds, but greenhouse- and field-grown sh2 were two to three times greater than su, respectively. Mature field-grown sh2 seeds leaked significantly more than greenhouse-grown sh2.

The trends of potassium leachability during seed development followed similar patterns as the total conductivity values, regardless of maturity or production environment (Figure 3-3). Conductivity measures the amount of electrolytes which include potassium. Both measurements correlated well in the present study (0.897 for sh2, 0.989 for su).

Total carbohydrates in the leachate generally decreased with increasing seed maturity in both genotypes (Figure 3-4). Significantly greater amounts of carbohydrates were lost from greenhouse-grown sh2 seeds 18 to 34 days post-pollination than from field-grown sh2 during the same period, with both types exhibiting a peak at 30 days after pollination. Soluble carbohydrate levels were significantly greater from greenhouse-grown than from field-grown su seeds 18 to 38 days post-pollination. Greenhouse-grown su seeds generally lost equivalent amounts of carbohydrates as field-grown sh2 throughout development. At maturity (42 to 46 days post-pollination), no significant differences were observed in amounts of soluble carbohydrates between all four seed types.

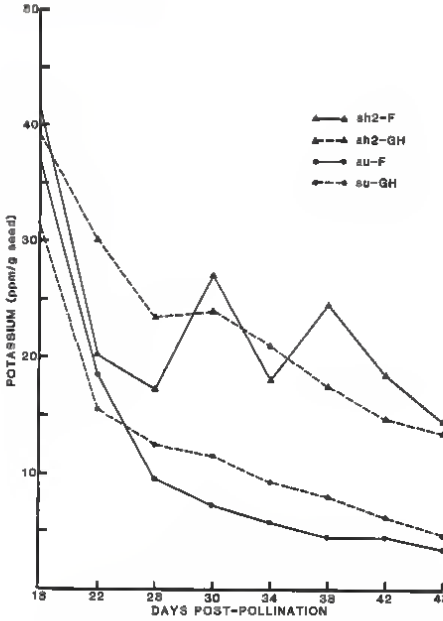


Figure 3-3 Potassium in leachate of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development

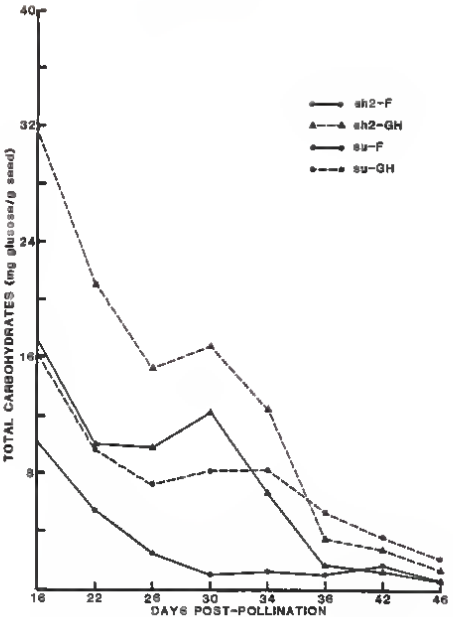


Figure 3-4. Total carbohydrates in leachate of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development

Significantly more sugars (fructose, glucose, and sucrose) leached from field-grown sh2 seeds than greenhouse-grown 18 to 26 days post-pollination (Figure 3-5). This leakage declined steadily with increasing seed maturity. Greenhouse-grown sh2 and field-grown sn seeds generally had low total sugars in the leachate throughout development, whereas no sugars leaked from greenhouse-grown su seeds. There were no significant differences in sugar leakage in seeds 38 to 46 days post-pollination. Generally, the amount of total sugars in the leachate did not account for the amount of total carbohydrates measured previously.

In order to determine differences in seed surface integrity, scanning electron micrographs were taken of whole sh2 and su seeds during development (Figures 3-6 and 3-7). The micrographs were taken from essentially the same location on each seed. An examination of the seed surface indicated no noticeable differences between sh2 and su, regardless of maturity. There was a general lack of diversity in cuticular appearance and an absence of cracks in the pericarp in all seeds examined.

Studies were made of the structure of the protective layers (pericarp and aleurone layer) of immature and mature sh2 and su seeds (Figures 3-8 and 3-9). After examining many specimens, mature seeds of sh2 and su appeared to have a thicker and denser pericarp than immature seeds, however, no measurements were taken. Generally, the pericarp in mature seeds of both genotypes adhered more tightly to the aleurone layer than in immature seeds, although not necessarily in all cases. The aleurone layer of su seeds appeared to be more dense than sh2, regardless of maturity. Greenhouse-grown seeds of

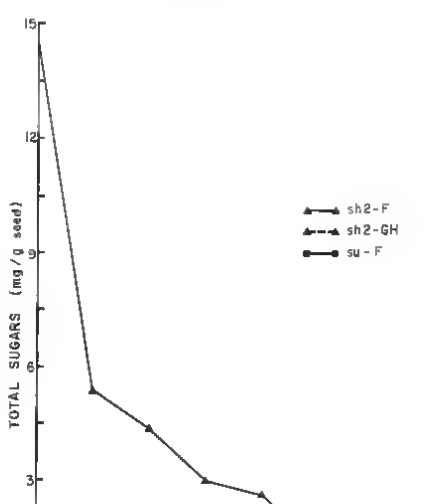


Figure 3-5 Amount of total sugars in leachate of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development (No measurable sugars were detected in leachate from greenhouse-grown su seeds)

Figure 3-6: Scanning electron micrographs of seed surfaces of 22-day-old *sh2* (A) and *su* (C) and 30-day-old *sh2* (B) and *su* (D)

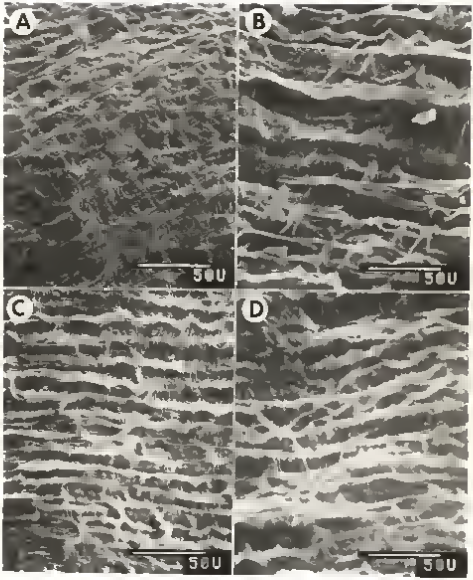

A
50U
B
50U
C
50U
D
50U

Figure 3-7. Scanning electron micrographs of seed surfaces of 38-day-old sh2 (A) and su (C) and 46-day-old sh2 (B) and su (D)

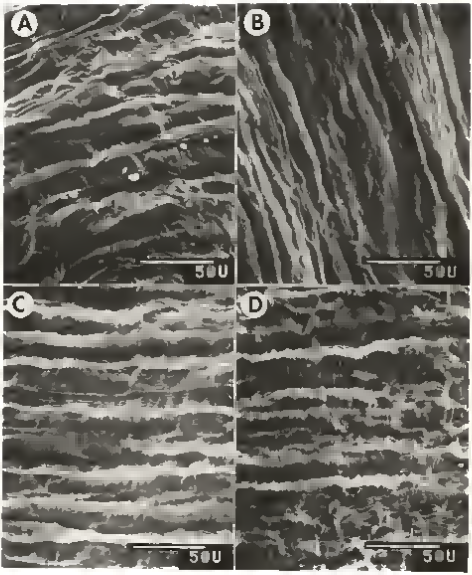

A
50U
B
50U
C
50U
D
50U

Figure 3-8. Light micrographs of pericarp (P), aleurone layer (Al), and endosperm (E) of immature (22-day-old) field-grown (A, C) and greenhouse-grown (B, D) seeds of sh2 (A, B) and su (C, D) x 1600

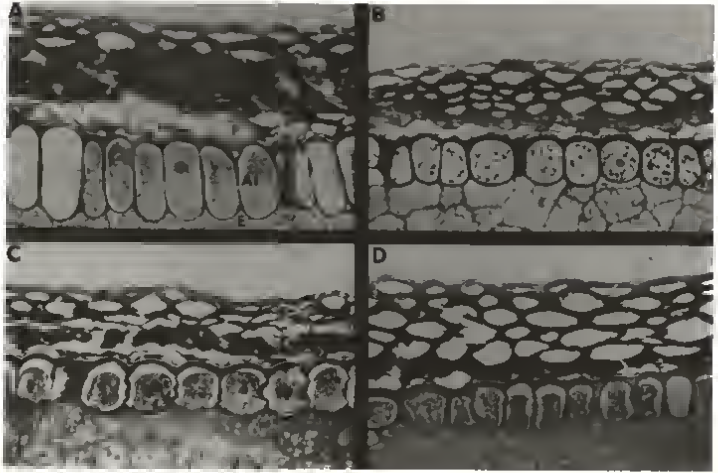
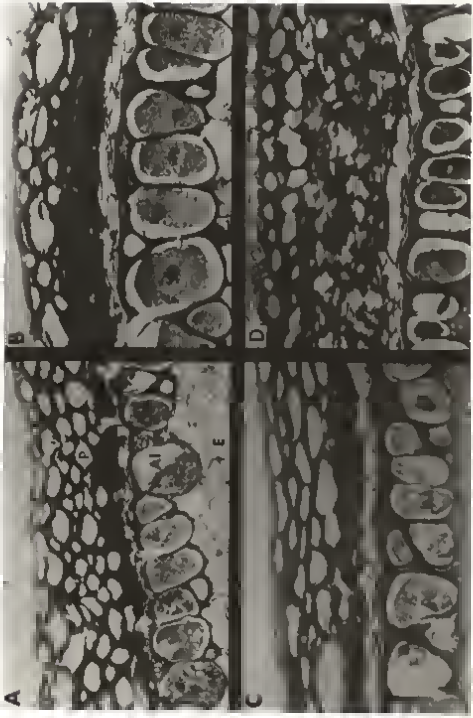

A
B
C
D

Figure 3-9. Light micrographs of pericarp (P), aleurone layer (Al), and endosperm (E) of mature (45-day-old) field-grown (A, C) and greenhouse-grown (B, D) seeds of *sh2* (A, B) and *su* (C, D) x 1600


A
B
C
D
E
P
N

either genotype seemed to have a thicker end sometimes denser pericarp than field-grown seeds, especially at the mature stage of development.

Seeds were taken from ears harvested at various stages of development and dried for 2 weeks at 30° C to approximately 8% moisture, after which they were ground to a powder, completely dried, and analyzed. Total carbohydrate levels of sh2 seeds were 20 to 35% less than su seeds throughout development (Figure 3-10, Table A-7). Greenhouse-grown sh2 seeds 22 to 46 days post-pollination contained lower amounts of carbohydrates than field-grown sh2 seeds, but significant differences occurred only at 26, 30, and 46 days after pollination. The highest carbohydrate levels in both field- and greenhouse-grown sh2 seeds were at 22 days, and declined from 22 to 30 days post-pollination. No overall increase in carbohydrate content from 18 to 46 days post-pollination was noted in field-grown sh2 and greenhouse-grown sh2 and su seeds. Field-grown su seeds contained significantly less carbohydrates than greenhouse-grown su seeds 18 to 30 days old. These differences, however, were reversed at 42 to 46 days post-pollination.

The quantities of starch in sh2 seeds throughout development were significantly less than in su seeds (Figure 3-11). Greenhouse-grown sh2 seeds contained significantly lower amounts of starch than field-grown sh2 18 to 46 days post-pollination. Concentrations of starch in su seeds continued to increase in field-grown samples during the entire period of development to 42 days post-pollination, but in greenhouse-grown seeds starch levels were essentially the same at 22 and 46 days post-pollination. At maturity, field-grown su seeds had

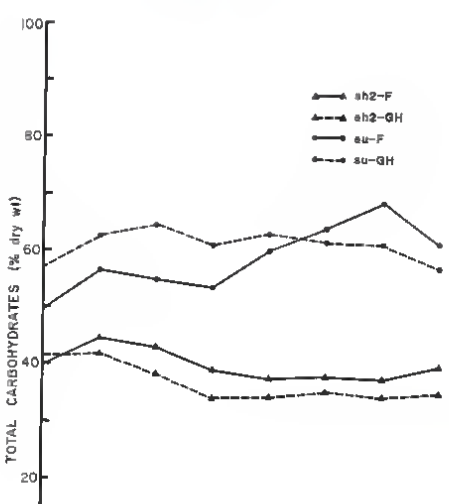


Figure 3-10 Total carbohydrates in field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30° C to 8% moisture

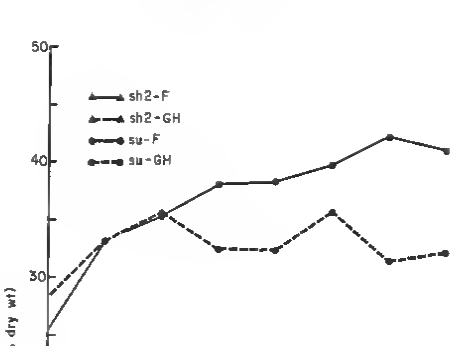


Figure 3-11. Amount of starch in field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30° C to 8% moisture

61% more starch than at 18 days post-pollination, whereas greenhouse-grown sh sweet corn seeds had only 14%.

Individual quantities of fructose, glucose, sucrose, and total sugars of field- and greenhouse-grown sh2 and su seeds during development appear in Figures 3-12 to 3-15. The amounts of fructose and glucose were much lower than sucrose in all seeds analyzed. Fructose levels continually declined with maturity, regardless of seed type (Figure 3-12, Table A-8). Seeds of sh2 had two to four times more fructose than su seeds 18 to 24 days post-pollination. Field-grown sh2 seeds contained significantly greater amounts of fructose than greenhouse-grown sh2 from 26 dyas after pollination until maturity. Significantly more fructose was measured in field- than greenhouse-grown su seeds 18 to 30 days post-pollination but not thereafter. The glucose content of field-grown sh seeds was significantly greater than the other three seed types throughout development (Figure 3-13). Field-grown sh seeds exhibited a peak in glucose levels at 30 days, while greenhouse-grown sh peaked at 34 days post-pollination. Although the amount of glucose was low in sh2 seeds during development, greenhouse-grown seeds contained no glucose after 34 days post-pollination.

Since sucrose comprised nearly all of the total sugars as measured in this study, the developmental patterns of sucrose and total sugars were very similar (Figures 3-14 and 3-15). Sucrose levels of sh2 seeds were almost two times greater than su seeds 18 to 26 days after pollination (Figure 3-14). Both sh2 types had a peak in sucrose content at 26 days, while field- and greenhouse-grown su peaked at 30 and 34 days post-pollination, respectively. Field-grown su seeds contained significantly more sucrose than greenhouse-grown sh 26 to 46 days

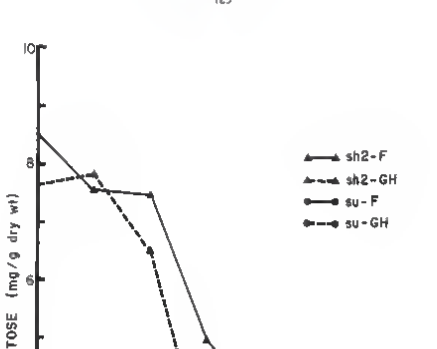


Figure 3-12. Amount of fructose in field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30° C to 8% moisture

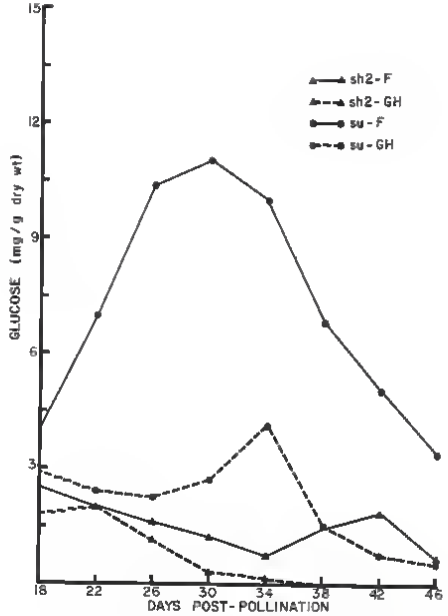


Figure 3-13 Amount of glucose in field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30° C to 8% moisture

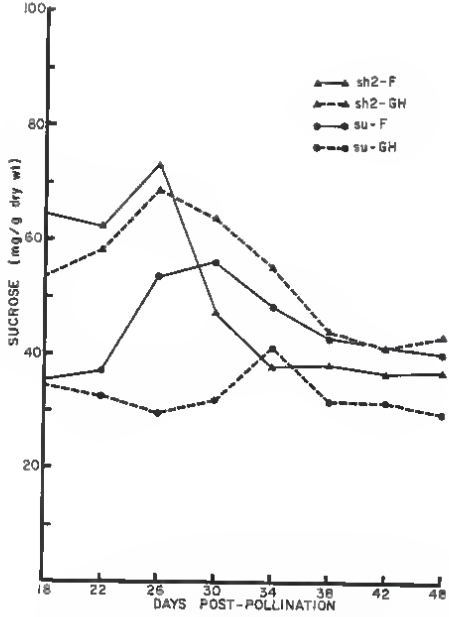


Figure 3-14. Amount of sucrose in field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30° C to 8% moisture

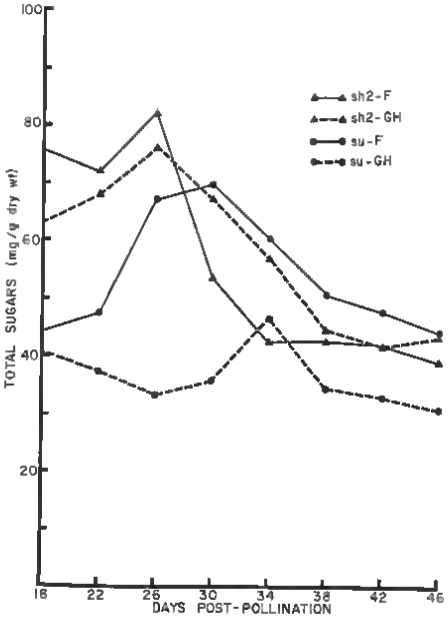


Figure 3-15. Amount of total sugars in field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30° C to 8% moisture

post-pollination. At maturity, no significant differences were noted in sucrose content between both sh2 types and field-grown su seeds. Determination of total sugar contents indicated that field-grown su and greenhouse-grown sh2 seeds 30 to 46 days old contained equal amounts of sugar (Figure 3-15).

Sugar and starch contents of immature and mature freeze-dried sh2 and sn kernels were compared to oven-dried (30° C) kernels to determine possible conversion of sugars to starch during the drying process and losses due to respiration (Table 3-1). Total sugar content of immature freeze-dried sh2 kernels was at least three times more than immature oven-dried sh2. All three sugars reflected this increase. The amount of starch in 22-day-old oven-dried sh2 kernels was greater than their freeze-dried counterpart. Mature oven-dried sh2 seeds contained similar quantities of sugars as freeze-dried sh2 but had lower amounts of starch. Freeze-dried immature sn kernels had twice as much sugar and considerably more starch than oven-dried sn kernels. At maturity, sugar contents of freeze- and oven-dried sn seeds were equal, but a higher starch level than oven-dried sn was still noted in freeze-dried su seeds.

Respiration rates of 46-day-old field-grown sh2 and sn seeds imbibed up to 96 hours were measured to determine if the carbohydrate reserves of sh2 were adequate for germination (Figure 3-16). Mature sh2 seeds respired at a greater rate than sn throughout the germination period. The respiration rate of sh2 seeds increased more than su during the first 60 hours of imbibition, during which radicle emergence occurred.

Table 3-1. Sugar and starch contents of oven- and freeze-dried, immature and mature, field-grown kernels of sh2 and su

Type	Date[Z]	Treatment	Sugars (mg/g dry wt)				Starch (mg/g dry wt)
			Fructose	Glucose	Sucrose	Total	
sh2	22	Oven-dried	7.5 ± 0.2[Y]	2.0 ± 0.1	62 ± 5	72 ± 5	287 ± 9
		Freeze-dried	33.7 ± 1.5	18.0 ± 1.4	194 ± 7	245 ± 10	106 ± 7
	38[X]	Oven-dried	3.1 ± 0.2	1.4 ± 0.1	38 ± 1	43 ± 2	166 ± 2
		Freeze-dried	1.3 ± 0.2	0.3 ± 0.06	53 ± 3	55 ± 3	190 ± 1
su	22	Oven-dried	3.3 ± 0.2	7.0 ± 0.4	37 ± 1	47 ± 1	332 ± 13
		Freeze-dried	10.2 ± 0.3	6.5 ± 0.2	74 ± 1	90 ± 1	458 ± 6
	46	Oven-dried	0.9 ± 0.07	3.4 ± 0.3	40 ± 2	44 ± 2	411 ± 15
		Freeze-dried	0.8 ± 0.18	2.0 ± 0.5	41 ± 1	44 ± 1	480 ± 3

[Z]Days post-pollination.

[Y]Standard error of mean.

[X]Selected as most mature stage because older ears of freeze-dried 1978 crop were destroyed by rain.

Figure 3-16 Respiration rates of mature (46-day-old) field-grown *sh2* and *su* seeds imbibed for various periods of time (Arrows indicate average time of radicle emergence.)

sh2
su
O_2 UPTAKE (µl/hr/seed)
280
200
120
40
20
40
60
80
100
IMBIBITION TIME (hours)

Discussion

The greater rate of imbibition throughout germination of mature sh2 seeds as compared with su may be attributed to several factors. The small seed size and weight of sh2 may allow more water to be absorbed within a period of time per unit area than the larger-seeded sn. Shiah and McDonald (1980) noted that small flat corn seeds had a faster rate of water uptake than large round seeds during the initial stages of germination. Another factor may be the sugar content of sh2 seeds. The higher levels of sugars in sh2 seeds could increase the osmotic potential, thus giving the potential for more water uptake during the initial phase of imbibition. Sugary seeds had a lesser sugar to starch ratio than sh2, resulting in a lower osmotic potential.

Structure and integrity of the outer protective layers of sh2 and su seeds can contribute to the observed difference in water uptake. These outer layers consist of the pericarp, seed coat, and aleurone layer. Relatively little water is absorbed into the seed through the pericarp because of cutinization of the outer surface of the pericarp (Wolf et al., 1952a). Breaks in the pericarp would greatly increase the movement of water through this region. Although no cracks in the pericarp of hand-harvested sh2 seeds were noted in this study, mechanical damage due to machine-harvesting of the seeds may occur. A greater amount of large elrspaces between the pericarp and aleurone layer were noted within mature sh2 seeds (personal observation). These spaces may allow the pericarp to be easily broken as well as facilitating water movement, even after the greatest care in handling.

Hinton (1955) determined that the seed coat or testa of wheat was the layer offering the greatest resistance to water entry. In corn, the

seed coat is a thin hyaline membrane, thus, the protective function is assumed by the pericarp (Wolf et al., 1952a). The true seed coat and aleurone layer of corn are semi-permeable, providing additional layers for water to penetrate (Wolf et al., 1952b). The aleurone layer of mature sh2 seeds appeared to be less dense and perhaps less lignified than in su. No observations were made on differences in the thin seed coat between sh2 and su. Generally, the protective layers of mature sh2 seeds were not as thick or dense as in mature su seeds, resulting in more water penetrating these layers, particularly if broken. The degree of lignification and thickness has been shown to affect the permeability of the seed coat in lima bean (Kannenburg and Allard, 1964) and snap bean seeds (Hyatt, 1977).

Production of mature sh2 and su seeds in the greenhouse influenced their imbibition rates as compared to production in the field. The significant differences noted after 1 to 24 hours of imbibition between field- and greenhouse-grown sh2 seeds can be attributed to factors previously mentioned. Greenhouse-grown sh2 seeds had a thicker and denser pericarp and less carbohydrates than field-grown seeds. These factors affected imbibition through radicle emergence which occurred at 36 hours. At this point, greenhouse-grown sh2 and su seeds imbibed significantly more water than field-grown seeds.

Most of the water entering the corn seed is taken up through the basal end of the tip cap and moves rapidly through the spongy parenchyma cells of this area and the inner pericarp (Wolf et al., 1952a). Water can then diffuse through the aleurone layer and basal endosperm cells, moving into the endosperm and embryo at different rates. Grush and Milner (1969) found that compositional differences in the endosperm of

wheat grains determined rate of water penetration. The reduced amount of endosperm of mature sh2 seeds, containing large airspaces and low amounts of tightly packed starch granules, partly accounted for the rapid hydration of these seeds. The corn embryo can preferentially increase in water content. Blacklow (1972) determined that when the water content of dent corn seed reaches 75%, the water content of the embryo on a dry weight basis is 261%, but that of the remainder of the seed is only 50%. Rapid water movement into sh2 seeds would result in accelerated hydration of the embryo, increasing the potential for more damage or leakage.

Imbibitional injury of sh2 seeds may be due to several factors. Cellular rupture during imbibition damaged legume seeds with breaks in their seed coats (Duke and Kakefuda, 1981). Greater injury can occur when seeds are imbibed under cold conditions. Tully et al. (1981) concluded that this injury was due to the rapidity of imbibition which is principally controlled by the seed coat. Reorganization of membranes may be disrupted by the inrush of water and cold temperatures (Bramlage et al., 1978, Parry and Harrison, 1970). In Chapter II, sh2 seeds performed poorly in the cold soil test. The rapid imbibition rate of sh2 seeds may be lethal under these conditions. Osmotic control of water uptake in the initial stages of germination by polyethylene glycol (PEG) may prevent imbibitional injury and increase vigor of sh2 seeds, as demonstrated in low-vigor embryonic axes of soybean (Woodstock and Tao, 1981).

The greater imbibition rate of sh2 seeds led to increased seed leakage. During development, sh2 seeds generally leaked more electrolytes and, to some extent, carbohydrates than su. This leakage declined

with maturity, as the protective layers of sh2 and su seeds became thicker and more lignified. Compositional changes during development, such as the increase in starch content in sh seeds, helped to reduce loss of soluble materials. At maturity, leakage of electrolytes was significantly greater from sh2 than su seeds. However, no significant differences were observed between sh2 and su in quantities of soluble carbohydrates and sugars leached. Sugar levels (fructose, glucose, and sucrose) did not account for the majority of soluble carbohydrates measured. The anthrone-sulfuric acid method used for carbohydrate determinations can measure mono-, di-, and polysaccharides, dextrins, dextrans, starches, gums, and glucosides (Umbreit et al., 1959). Apparently, carbohydrates other than the three sugars measured were leaking out of the seeds. Ethanol and acetaldehyde have been shown to increase in amounts during imbibition of aged and damaged soybean seeds (Woodstock and Taylorson, 1981). These compounds may also accumulate and become toxic during imbibition of infected pea seeds (G. E. Harman, personal communication).

Production environment eventually influenced seed leakage in several ways. Greenhouse-grown seeds of either genotype had thicker and denser pericarps than field-grown seeds at maturity. This difference in pericarp thickness reduced the imbibition rate of mature greenhouse-grown sh2 seeds, thereby reducing leakage of electrolytes. However, more carbohydrates but less sugars leaked from greenhouse-grown seeds of sh2 and su compared with field-grown seeds until maturity. Matthews and Rogerson (1976) felt that differences in leaching of solutes from different seed lots of peas were associated with the condition of the embryos and not the seed coats. In addition, these differences were

related to the ability of the embryos to retain solutes rather than their initial solute content. With greenhouse-grown sh2 seeds, the initial carbohydrate contents were less than or equal to field-grown sh2 throughout development. On the other hand, the differences in seed vigor between field- and greenhouse-grown sh2 and su (Chapter II) did not agree with carbohydrate leakage, but were more closely depicted by conductivity measurements.

Seeds produced in the field were exposed to and infected by pathogens at some point during development (Chapter IV). After harvesting and drying, infected sh2 seeds leaked more electrolytes and sugars than noninfected greenhouse-grown sh2. Infection during seed development may alter the seed's ability to retain solutes and thus rapidly germinate. Harman and Granatt (1972) determined that solute leakage from pea seeds inoculated with Aspergillus ruber was greater than from noninoculated seeds, although the rate of increase in leakage became similar shortly after infection began. This difference in leakage was attributed to plasmalemma damage and occurred in both aged and infected seeds.

Emergence in the field of seeds that leak heavily can be seriously reduced. In Chapter II, sh2 seeds emerged poorly in the cold soil test, and have been shown to leak more electrolytes and other solutes than su seeds throughout development, regardless of the production environment. The result of this leakage could be increased pathogenic attack in the soil. Field conditions, such as cold and wet soils, can delay seed germination and increase seed exudation (Schroth et al., 1966). Substances leaking out of pea seeds into the soil stimulated the germination and growth of fungal pathogens in the soil, producing seed and

seedling rot (Schroth and Cook, 1964, Short and Lacy, 1974). These substances were usually sugars and amino acids. Pea seeds susceptible to seed rot exuded three times more soluble carbohydrates (fructose, glucose, and largely sucrose) than nonsusceptible seeds (Loria and Lacy, 1979). Compounds such as ethanol and acetaldehyde may also be important in seed leakage and soil pathogen stimulation (G. E. Harman, personal communication).

High levels of leakage have been observed in aged soybeans (Woodstock and Tao, 1981), infected peas (Harman and Granett, 1972), immature peas (Matthews, 1973), and bleached pea seeds (Loria and Lacy, 1979), and were positively correlated with loss of seed vigor. Perry and Harrison (1970) found that more electrolytes exuded from dead and low-vigor pea seeds than from high-vigor seeds. Leakage was negatively correlated to field emergence of peas and French beans (Matthews and Bradnock, 1978). In the present study, leachate conductivity appeared to agree with seedling vigor measurements in Chapter II in accurately characterizing vigor differences between sh2 and su during development, regardless of production environment. The high amount of leakage associated with sh2 seeds may seriously deplete the reserves of the endosperm and especially the embryo, resulting in reduced germination and vigor and increased susceptibility to soil pathogens under field conditions.

The carbohydrate reserves of whole dry seeds of sh2 and su were altered by production environment. However, seed vigor was not effected by these changes. Greenhouse-grown sh2 seeds were heavier (Chapter II), but contained less starch than field-grown seeds throughout development. Quantities of carbohydrates and sugars were similar, although leakage of

carbohydrates was greater before maturity in greenhouse-grown sh2 seeds. Viability and vigor of these seeds during development, and especially at maturity, were better than that of field-grown sh2 seeds (Chapter II). Production of su seeds in the greenhouse reduced the carbohydrate, starch, and sugar contents, particularly at maturity, when compared with field-grown su. In addition, there were no overall increases during development in the amounts of carbohydrates and starch of greenhouse-grown su seeds. The environment of the greenhouse may not be forcing the kernels to produce and store carbohydrates to the same extent as the field environment does. Reduced light conditions in the greenhouse, particularly in the fall and winter, may limit the amount of photosynthates available to the kernels and starch synthesis (Mengel and Judel, 1981).

The high levels of sugars of sh2 seeds during development not only affect imbibition and leakage, but also may be responsible for pathogenic infection of developing ears in the field. Previous work has shown that sh2 kernels contained three times more sugars than su 28 days post-pollination (Creech, 1965). At physiological maturity, sh2 had four times more sugars than su (Laughnan, 1953). These high sugar levels, combined with the high kernel moisture they maintain (Chapter II), would provide ideal conditions for infection and growth of pathogens. In the present study, sugar determinations of dry seeds did not fully indicate the large differences between sh2 and su throughout development, however, sugar levels of freeze-dried kernels did.

Comparison of sugar contents of freeze- and oven-dried sh2 and su kernels indicated that immature kernels lost sugars during the 2-week drying process. Immature freeze-dried su and sh2 kernels contained two

and three times, respectively, more sugars than oven-dried kernels. A possible conversion of sugars to starch was noted in immature oven-dried sh2 kernels. However, this possibility is unlikely due to the low activity of ADP-glucose pyrophosphorylase, a starch-forming enzyme, in sh2 (Dickinson and Preiss, 1966). A more plausible explanation would be an interaction with production environment, as greenhouse-grown sh2 samples (Figure 3-11) contained less starch than freeze-dried ones. Immature oven-dried su kernels did not have a large increase in starch. At maturity, sugar levels were equal in fresh and dry sh2 and su kernels while starch levels were less for oven-dried kernels. During drying, sugars may have been absorbed back into the cob in immature samples or converted to products other than starch.

Kernel respiration during the drying process may account for some of the sugar loss, as well as differences in starch contents. As the ears were drying at 30° C, respiration of sh2 and su kernels with high moisture contents and readily available sugars could increase to high rates for a period of time. Starch breakdown in su kernels could occur and these sugars, together with sugars already present, may be utilized to maintain a high respiratory rate. The full extent to which this process occurs and possible changes within immature kernels is not known. However, Knittle and Burris (1976) determined that respiration of fresh corn kernels decreased throughout maturation and coincided with moisture content and maximum kernel dry weight. Fussell and Pearson (1980) found that respiration and moisture content of detached pearl millet grains declined at a faster rate at high temperatures (33° C). They believed that any deleterious effects of high respiration rates on seed reserves were compensated by the reduced period of high respiration

The small endosperm and reduced carbohydrate content of mature sh2 seeds may adversely affect their germination and vigor. An early depletion of endosperm reserves could cause a reduction in seedling growth, leaving the seedling vulnerable to pathogenic attack in the field. Respiration rates of mature field-grown sh2 seeds imbibed for up to 96 hours were always greater than their su counterparts. Determinations of vigor under optimum and stress conditions indicated that mature sh2 seeds were less vigorous than su (Chapter II). These results agree with the work of Wann (1980) who found that elevated respiration rates of sh2 seeds could not be correlated with seedling growth. Styer et al. (1980) obtained higher ATP levels for sh2 than su seeds germinated up to 96 hours. Apparently, the composition of sh2 seeds was more than adequate for respiration and energy production. Wahab and Burris (1971) suggested that high-quality soybean seeds were better able to mobilize and interconvert sugars to utilizable metabolites than low-quality seeds. In corn, the early germination growth of the embryonic axis was dependent upon the scutellar, rather than the endosperm, food reserves (Dure, 1960a). Infection by pathogens, particularly in sh2 seeds, may interfere somehow with embryonic growth and development.

Summary

Field- and greenhouse-grown seeds of sh2 and su were harvested 18 to 46 days post-pollination. Imbibition rate, seed structure, and carbohydrate composition of dry seed were analyzed to determine their relationships with seed leakage, vigor, and possible pathogenic attack.

Mature sh2 seeds had a greater imbibition rate than su for the first 60 hours of germination. After 22 days post-pollination, sh2

seeds leaked significantly more electrolytes, including potassium, than su seeds. Leachate conductivity was greater from field-grown sh2 seeds than greenhouse-grown sh2 at maturity. More carbohydrates were lost from greenhouse- than field-grown seeds 18 to 34 days post-pollination of both genotypes. No difference in carbohydrate leakage was observed between genotypes at maturity. Leakage generally decreased in all seed types with increasing maturity. Sugar levels (fructose, glucose, sucrose) did not account for the majority of carbohydrates in the leachate.

Cracking of the outer seed surface (pericarp) was not noticeable in either sh2 or su during development. In mature seeds of either genotype, the pericarp appeared to be thicker and adhered more tightly to the aleurone layer than in immature seeds. Mature greenhouse-grown seeds of sh2 and su had a denser pericarp than field-grown seeds. The aleurone layer in su seeds was possibly more lignified than in sh2 seeds, regardless of maturity.

Dry seeds of sh2 contained considerably less total carbohydrates and starch than su 18 to 46 days post-pollination. Field-grown su seeds accumulated more carbohydrates during development than the other seed types. Greenhouse-grown sh2 seeds had a lower starch content than field-grown sh2. Immature (18 to 26 days post-pollination) sh2 seeds had significantly higher sugar contents than su at the same developmental stage. The lowest amounts of sugars in dry seeds occurred at maturity with no difference noted between field- and greenhouse-grown sh2 and field-grown su.

Immature fresh kernels (freeze-dried) of su and sh2 contained two and three times, respectively, more sugars than immature dry kernels

(oven-dried at 30° C for 2 weeks). At maturity, fresh and dry kernels of either genotype had equal amounts of sugars, but fresh kernels contained more starch. Although immature dry sh2 kernels had more starch than fresh kernels, this difference was probably due to environment and not to conversion of sugars to starch. Respirational loss of sugars at the immature stage might have occurred, particularly in sh2. Respiration rates of mature imbibed seeds indicated that the quantity and composition of endosperm of sh2 was more than adequate for germination and early seedling growth.

In conclusion, the high imbibiton rate of sh2 seeds was due to their small size, high sugar to starch ratio, and thinner seed protective layers. Leakage of sh2 seeds was probably increased by the imbibition rate and the above mentioned factors. Generally, leakage of both genotypes declined with maturity because of structural and compositional differences. Increases in leachate conductivity agreed with previous determinations of poor seed vigor. The greenhouse environment produced seeds that (1) had thicker pericarps at maturity, (2) had reduced carbohydrate reserves, (3) leaked more carbohydrates but less sugars, and (4) had greater seed vigor than field-grown seeds, particularly in sh2. The lack of pathogens associated with the greenhouse-grown seeds may be responsible. Seed leakage might increase the potential for pathogenic attack of sh2 in the soil.

CHAPTER IV
ASSOCIATION OF Fusarium moniliforme WITH SEED VIGOR DURING SEED DEVELOPMENT OF TWO ENDOSPERM MUTANTS OF SWEET CORN

Fusarium moniliforme commonly infects a wide range of crops throughout the world and is a major parasite of members of the Graminae, particularly in tropical and subtropical regions (Booth, 1971). This fungus can cause stalk rot, one of the most destructive diseases of corn throughout the world (Christensen and Wilcoxson, 1966; Shurtleff, 1977). Yield losses of 10 to 20% from stalk rot alone are common in the United States (Shurtleff, 1977). In addition, F. moniliforme can cause leaf spot, ear and kernal rot, seed rot, damping-off, and seedling blight (Shurtleff, 1977; Smith and Madsen, 1949; Warren, 1978). Although F. moniliforme is known to cause kernel rot, the pathogen is often associated with kernels that appear not to be diseased or damaged (Koehler, 1942; Thomas and Buddenhagen, 1980). Planting infected seed may increase the incidence of seedling blight (Futrell and Kilgore, 1969) and contribute to systemic infection of plants (Foley, 1962). However, the importance of infected seed as a source of subsequent plant infection seems minimal, because seedlings are readily infected by F. moniliforme from infested soil debris (Kucharek and Kommedahl, 1966). Usually, sweet corn is more susceptible than field corn to seed rot and seedling blight (Shurtleff, 1977).

The time of entry of F. moniliforme into corn kernals is not clearly understood. Seed infections with F. moniliforme were present in

the milk stage and the percentage of infection increased progressively until the mature stage (Koehler et al., 1934). Fusarium spp. were isolated from kernels the third week after silk emergence and had a peak occurrence in the eighth week (Hesseltine and Bothast, 1977). King (1981) first isolated F. moniliforme 2 weeks after midsilk and infection increased to 35 to 66% by the tenth week.

Maturing corn kernels may be infected either by transmission from outside or systemically from the plant. Koehler (1942) found that F. moniliforme generally entered in the region of the silks and the kernels became contaminated in contact with the silks. The infection then spread to the pedicels, vascular cylinder, and finally the shank. Kernel infection in the tip half of corn ears before maturity was most common (King, 1981). Helminthosporium maydis ear rot predisposed corn seed to invasion by F. moniliforme by providing access into the ear (Doupnik, 1972). Salama and Mishricky (1973) suggested that immature seeds became infected with F. moniliforme through the tip cap and placento-chalazal region. Lawrence et al. (1981) determined that the pathogen grew from infected kernels upward in the plant to the cob where it infected developing kernels.

Location of F. moniliforme in the corn seed itself can vary. Corn seeds can be infested and infected with F. moniliforme. El-Helaify et al. (1981) suggested that the pathogen was primarily a surface contaminant occurring in cracks and natural openings in the pericarp. Salama and Mishricky (1973) were able to detect fungal mycelium in internal tissues of mature corn seeds. However, Lawrence et al. (1981) found not hyphae but spherical to amorphous bodies throughout the tip cap and occasionally in the endosperm between the tip cap and embryo.

Insects, such as corn earworms or borers, may aid in the development of stalk and ear rots by F. moniliforme by carrying inoculum into tissues and causing wounds through which the pathogen can enter (Christensen and Wilcoxson, 1966, Koehler, 1960, Shurtleff, 1977, Smeltzer, 1959). Numerous methods have been used to artificially inoculate corn with ear-rotting fungi. Some are aerially disseminated while others simulate insect damage (Boling et al., 1963, Gulya et al., 1980, Loesch et al., 1976, Ullstrup, 1970). A high incidence of infection was observed when ears were inoculated by spraying silks 6 to 18 days post-pollination (Warren, 1978). Inoculation made after 22 days resulted in only small amounts of kernel rot.

Germinating corn kernels may be attacked by a number of soil- or seed-borne fungi, including F. moniliforme, which cause seed rots and seedling blights. Severe infection may kill the embryo before germination (seed rot) or destroy the seedling before or after emergence (Shurtleff, 1977). A seedling blight of corn was caused by F. moniliforme and the mode of action of the fungus was by a heat-stable and completely water-soluble toxin which inhibited root growth (Futrell and Kilgore, 1969, Scott and Futrell, 1970). Fusarium moniliforme produced a toxin in vitro that had a deleterious effect on both plants and animals (Cole et al., 1973). Symptoms of abnormality and reduced growth of uninfected corn embryos were found by Brodink (1975) to be due to toxic metabolites of F. moniliforme.

Sweet corn hybrids containing the mutant gene shrunken-2 (sh2) in place of the standard sugary (su) gene had two to three times more sugar and one-third less starch 16 to 28 days post-pollination (Creech, 1965). The higher sugar content of the sh2 kernels during development has been

associated with an increase in rot by pathogens during germination (Berger and Wolf, 1974, Pieczarka and Wolf, 1978). Genotypic differences in susceptibility have been reported for F. moniliforme kernel rot and asymptomatic kernel infection (Smith and Madsen, 1949, Warren, 1978). Inbred differences for asymptomatic kernel infection were expressed in their hybrids (King and Scott, 1981).

The following experiments were designed to determine (1) the interaction of F. moniliforme with two endosperm mutants of sweet corn during seed development, and (2) the effects of this pathogen on subsequent seed viability and vigor.

Materials and Methods

Seeds of sh2 and su were produced in the field and greenhouse and harvested at various developmental stages (18 to 46 days post-pollination) as previously described in Chapter II.

Fungal Infection

Seeds of field- and greenhouse-grown sh2 and su were surface sterilized with 1.8% sodium hypochlorite for 5 minutes and rinsed three times with sterile distilled water. Ten replications of five seeds were plated on acidified potato dextrose agar (PDA) media and incubated for 7 days at 25° C under continuous light. Fungal-infected seeds were counted and expressed on a percentage basis.

Entrence of Pathogen into the Ear

Twenty-five seeds were randomly selected from at least five tip and butt halves of ears of 1978 field-grown sh2 and su. All seeds were

surface sterilized, plated, and incubated as previously described. After 7 days, only seeds infected by Fusarium moniliforme were counted.

Greenhouse Inoculation

Ears of sh2 and su were inoculated with F. moniliforme in the greenhouse 10 and 20 days after pollination. Inoculations were made at the silk end by injecting 0.5 ml of a 10^6 spores/ml suspension into the silk tuft (Lillehoj et al., 1976). The spore suspension was prepared by adding 5 ml of sterile water containing one drop of Triton-X per 100 ml to five 2-week-old PDA plate cultures. The suspensions were combined and adjusted to the final concentration with a hemacytometer.

Four ears of each seed type and inoculation time were harvested 30, 38, and 46 days post-pollination. In addition, four control ears of 46-day-old sh2 and su injected with sterile water plus Triton-X only were harvested. All ears were husked, dried for 2 weeks at 30° C, and rated for ear rot. Ear rot rating was on a scale of 1 to 5, with 1 having no rot and 5 having greater than 75% rot. Visibly noninfected seeds were removed from the cob. Fifty seeds per ear were surface sterilized for 5 minutes in 1.8% sodium hypochlorite, rinsed three times in sterile distilled water, and plated on acidified PDA. Plates were incubated as before and number of F. moniliforme-infected seeds counted.

Location of Fungi in Seeds

Ten seeds each of field-grown sh2 (30 and 46 days old) and su (38 and 46 days old) and inoculated greenhouse-grown mature sh2 and su were individually soaked in water for 24 hours at 25° C. The pericarp, tip cap, endosperm, and embryo were dissected aseptically, surface sterilized

for 2 minutes in 1.8% sodium hypochlorite, rinsed three times in sterile distilled water, and plated on acidified PDA, one seed per plate (Mathur et al., 1975). All plates were incubated for 7 days at 25° C under continuous light and seed parts infected with F. moniliforme were determined.

Physiological Comparison of Infected and Noninfected Seeds

Mature infected (greater than 50% of total seeds) and noninfected greenhouse-grown sh2 and su seeds were tested for germination and vigor under optimum (25° C) and cold soil conditions (Chapter II). In each test, five replications of 20 seeds each were conducted. Leachate conductivity and total carbohydrates were determined on four replications of 25 seeds as previously outlined in Chapter III. Respiration rates of four replications of five seeds imbibed for 4, 12, 24, 36, and 48 hours were measured as in Chapter III.

Embryo Growth with Toxin

Toxic products of F. moniliforme were extracted by grinding approximately 300 g of mature infected field-grown su seeds and autoclaving in a large flask (Broduik, 1975). Six hundred milliliters of sterile distilled water were added and the flask was incubated at 10° C overnight. After incubation, the slurry was passed through sterile gauze, adjusted to 900 ml volume, and autoclaved.

Seeds of mature noninfected greenhouse-grown sh2 and su were surface sterilized for 5 minutes in 1.8% sodium hypochlorite and rinsed three times with sterile distilled water. Eighty seeds of each type were placed in sterile petri dishes with filter paper and imbibed for

48 hours at 25° C with 25 ml of either sterile distilled water or toxin extract. Four replications of 10 embryos were excised and sterilized for 1 minute in 1.8% sodium hypochlorite, rinsed three times in sterile distilled water, and plated on a medium consisting of either 2.5% sucrose and 1% agar, or 2.5% sucrose, 1% agar, and toxin extract (1:1 vol). The embryos were incubated for 5 days at 25° C and germination, numbers of abnormal seedlings, radicle and seedling lengths, and fresh and dry seedling weights were measured.

Scanning Electron Microscopy

Seeds for scanning electron microscope observations were freeze-dried and mounted on aluminum stubs with Tube Coat (G. C. Electronics Co., Rockford, IL). The samples were oven-dried at 70° C overnight and sputter-coated in a Technics Hummer V specimen coater with 60 nm of gold-palladium. Specimens were viewed in a Hitachi scanning electron microscope (SEM), Model S-450, using an accelerating voltage of 20 KV. Seed cross-sections were prepared by halving seeds with a single edge razor blade. Infected seeds were obtained by placing on cultures of F. moniliforme for 2 days, removed, then treated as above.

Results

The incidence of fungal infection in the field was considered severe in developing sh2 seeds almost 2 weeks earlier than in su seeds (Figure 4-1). More than 50% of the sh2 seeds were infected after 30 days post-pollination. Sugary seeds did not reach this high a level until after 42 days post-pollination. Seeds of sh which were produced in the greenhouse were not infected. Up to 60% of fully mature sh2

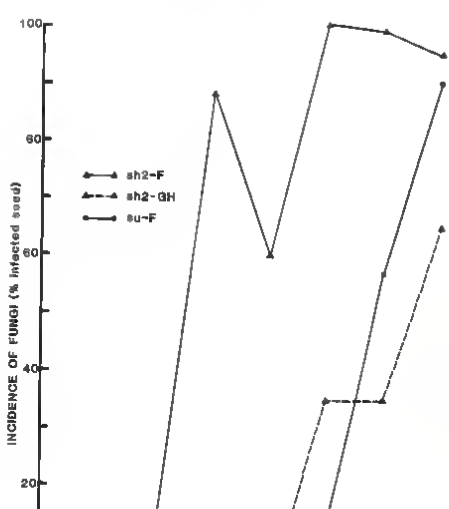


Figure 4-1. Incidence of fungal infection on field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development (No infection of greenhouse-grown su seeds was noted.)

seeds were infected when plants were grown in the greenhouse during late spring through early fall. However, mature noninfected sh2 seeds could be obtained when produced at other times of the year (data not shown). Different species of fungi were isolated from seeds produced in the two environments. Field-grown seeds were infected predominately with Fusarium moniliforme and a low incidence of Aspergillus flavus. When infected, greenhouse-grown seeds had primarily Penicillium spp. and no incidence of F. moniliforme.

Infection by F. moniliforme was generally greater in seeds from the tip half of ears of sh2 and su grown in 1978 (Table 4-1). No infection was recorded in seeds of su before 34 days, with high infection levels at 42 days post-pollination. Infection in sh2 seeds began at 25 days, but greatly increased after 30 days post-pollination. No sh2 ears were available 42 to 46 days after post-pollination because heavy rains and wind destroyed the remaining plants.

When harvested 26 to 28 days after inoculation, greenhouse-grown sh2 ears had significnatly more rot than noninoculated ears (Table 4-2). Ears of sh2 inoculated 20 days post-pollination had increasing amounts of rot as they matured. All inoculations of sh2 ears produced ear rot, indicating that the inoculum concentration was high. To compensate for this high concentration, only visibly noninfected seeds from outside the rotted areas on the ear were selected to study the spread of the pathogen. The highest percentages of seed infection were obtained from inoculated sh2 ears that had the greatest ear rot rating. Few significant differences in percentages of infected seeds were noted among sh2 ears, regardless of inoculation and harvest date. The low number of ears inoculated and variation within treatments resulted in the lack of

Table 4-1. Infection by *Fusarium moniliforme* of seeds from tip and butt halves of field-grown *su* and *sh2* ears harvested at various stages of development in 1978

Days post-pollination	Infected seeds (%)			
	su		*sh2*	
	Tip	Butt	Tip	Butt
18	0	0	0	0
22	0	0	0	0
26	0	0	0	12
30	0	0	36	8
34	0	4	36	44
38	0	4	88	80
42	48	28	--[z]	--
46	84	72	--	--

[z]No ears available because heavy rains and wind destroyed the remaining plants.

Table 4-2. Infection by Fusarium moniliforme of greenhouse-grown sh2 and su seeds from ears inoculated and harvested at various stages of development

Type	Inoculated[z] (days PP)	Harvested (days PP)	Ear rot rating[y]	Infected seeds (%)
sh2	10	30	1.6bc[x]	26ab
		38	3.3a	70a
		46	2.0bc	30ab
	20	30	1.3c	25ab
		38	1.8bc	12b
		46	2.7ab	40ab
	Noninoculated	46	1.0c	0b
su	10	30	2.0ab[x]	13ab
		38	2.5a	45a
		46	2.3a	45a
	20	30	1.0b	38ab
		38	2.0ab	25ab
		46	2.3a	52a
	Noninoculated	46	1.0b	0b

[z]PP = post-pollination.

[y]Ear rot rating was on a scale of 1 to 5, with 1 = no rot and 5 = 75% or greater rot.

[x]Mean separation in columns by Duncan's multiple range test, 5% level.

significance in seed infection. However, all inoculations did produce some F. moniliforme-infected seeds.

Significantly greater levels of ear rot were measured in sn ears about 4 weeks or more after inoculation compared with noninoculated ears (Table 4-2). No ear rot was observed 10 days after inoculation, but 38% of the seeds were infected. Seed infection percentages from sn ears inoculated 10 or 20 days post-pollination were not significantly different between harvest dates. Ears of sn that had the most ear rot also had a significantly greater number of infected seeds than noninoculated ears.

Entrance of F. moniliforme into the seed was determined by scanning electron microscopy of inoculated mature sh2 seeds (Figure 4-2). Hyphae with prolific microconidial production were prominent on the seed surface (Figure 4-2A). A macroconidium germinated on the seed with several hyphae originating from the spore (Figure 4-2B) and microconidia were evident but had not germinated. The collapsed appearances of hyphae and spores were due to desiccation by the freeze-drying treatment. Hyphae were seen entering through very small cracks in the pericarp (Figure 4-2C), but could also form appressoria to directly penetrate the seed surface (Figure 4-2D).

After penetrating the sh2 pericarp, the hyphae began growing throughout the area between the pericarp and aleurone layer (Figure 4-3). Due to the collapsed nature of sh2 seeds, the pericarp pulled away from the aleurone layer and formed small pockets, into which the hyphae were able to grow and sporulate. These pockets were not seen in the sn seeds.

The pericarp, tip cap, endosperm, and embryo of known infected groups of sh2 and su seeds were aseptically isolated to locate

Figure 4-2. Scanning electron micrographs of seed surface of matura (46-day-old) sh2 inoculated with Fusarium moniliforme

(A) Hyphae and microconidia
(B) Germinating macroconidium
(C) Hyphae entering through small crack in seed surface
(D) Hyphae directly penetrating seed surface by formation of appressoria

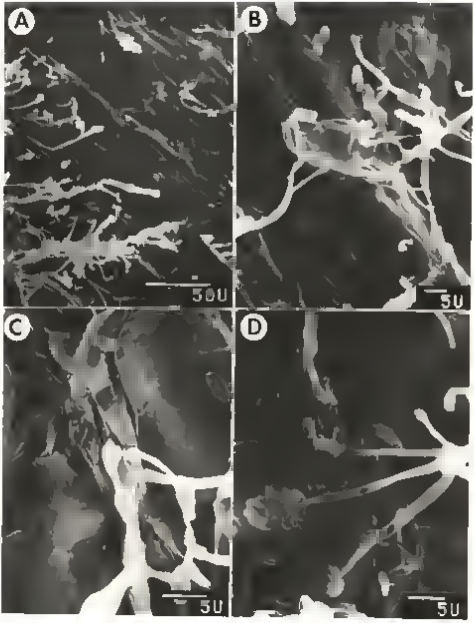
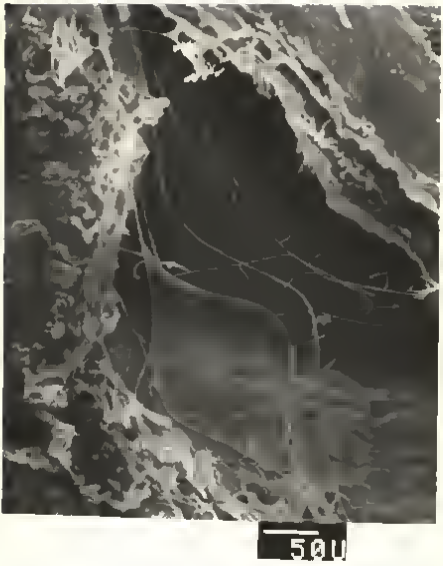

A
50U
B
5U
C
5U
D
5U

Figure 4-3 Scanning electron micrograph of crack between pericarp (above right) and aleurone layer (left and bottom) of a mature (46-day-old) sh2 seed inoculated with Fusarium moniliforme (Hyphae can be seen throughout this area.)


50µ

F. moniliforme within the seeds (Table 4-3). Although seed parts were surface sterilized for only 2 minutes, the sterilization time appeared to be too long to isolate the fungus from the pericarp and some tip caps. These seed parts are the primary areas for fungal entrence. Seeds with infected tip caps also had infected endosperm and embryos. These deep-seated infections were noted in mature (46-day-old) field-grown sh2 and su and inoculated greenhouse-grown sh2 and su seeds. The fungus was not isolated from embryos or endosperms of field-grown 38-day-old su seeds, but was isolated from embryos of 30-day-old sh2 seeds. These latter two seed ages were representative of the increase during seed development in field infection of sh2 and su by F. moniliforme.

Infection by F. moniliforme during seed development effected seedling vigor of mature greenhouse-grown sh2 and su seeds germinated under optimum conditions (Table 4-4). Germination percentages and rates were not significantly lower for infected seeds compared with noninfected seeds of either genotype. However, an increased number of abnormal seedlings was noted for infected seeds of su. Radicle and whole seedling lengths were significantly shorter for infected sh2 seeds. Seedling fresh and dry weights of infected su seeds were significantly less than those of noninfected seeds, whereas only seedling fresh weights were significantly different between sh2 infected and noninfected seeds.

Viability but none of the vigor indices of infected sh2 seeds in cold soil were significantly reduced compared with noninfected sh2 (Table 4-5). No differences were noted between infected and noninfected su seeds. Contrary to results during optimum germination tests, noninfected sh2 seeds were significantly less viable and vigorous than noninfected su seeds when subjected to cold soil conditions.

Table 4-3. Location of Fusarium moniliforme in isolated seed parts of sh2 and su seeds

Type[z]	Days post-pollination	Pericarp	Tip cap	Endosperm	Embryo
sh2 F	30[y]	-[x]	-	-	+
	46	-	+	+	+
su F	38[y]	-	-	-	-
	46	-	-	+	+
sh2 GHI	46	-	+	+	+
su GHI	46	-	+	+	+

[z]F = field-grown, GHI = greenhouse-grown inoculated.

[y]Developmental stage at which field infection of seeds by the pathogen increased.

[x]- = no fungus, + = presence of fungus.

Table 4-4. Optimum viability and vigor of infected (I) and noninfected (NI) mature greenhouse-grown sh2 and su seeds

Type	Germination (%)	Abnormal seedlings (%)	Germination rate index	Radicle length (cm)	Seedling length (cm)	Seedling wt	
						Fresh (g)	Dry (mg)
sh2 I	88ab[Z]	18ab	4.7ab	14.2b	22.9b	674c	96c
sh2 NI	96a	10b	5.2a	17.7a	28.0a	814b	97c
su I	93ab	25a	4.7ab	17.7a	27.2a	870b	137b
su NI	84b	9b	4.3b	18.4a	29.5a	971a	144a

[Z]Mean separation in columns by Duncan's multiple range test, 5% level.

Table 4-5 Cold soil viability and vigor of infected (I) and noninfected (NI) mature greenhouse-grown sh2 and su seeds

Type	Emergence (%)	Germination (%)	Abnormal seedlings (%)	Emergence rate index	Radicle length (cm)	Seedling length (cm)
sh2 I	13c[2]	21c	12b	0.3b	5.5b	12.6b
sh2 NI	23b	34b	18b	0.5b	6.7b	13.2b
su I	70a	87a	29a	1.5a	12.7a	21.5a
su NI	70a	89a	28a	1.6a	13.0a	22.0a

[2]Mean separation in columns by Duncan's multiple range test, 5% level

Leachate conductivity was slightly higher, but not significantly different, in noninfected than infected sh2 and su seeds (Table 4-6). Soluble carbohydrates which leached from noninfected su seeds were not greater than infected su, but almost twice the amount of carbohydrates leaked from noninfected sh2 seeds than from infected seeds. Seed leakage, as measured by conductivity and carbohydrates, was significantly greater in sh2 than su.

Infected seeds of both genotypes respired at significantly faster rates than noninfected seeds after 48 hours of imbibition (Table 4-7). By this time, radicle emergence had occurred. Noninfected sh2 seeds had significantly greater respiration rates than noninfected su seeds throughout the 48-hour imbibition period.

The toxic effect of F. moniliforme on growth of sh2 and su embryos was observed on sucrose-agar medium containing a suspension from infected su seeds (Table 4-8). A slight increase in the number of abnormal seedlings was noted after embryos, especially su, were exposed to toxin. Radicle growth of sh2 and su embryos was significantly inhibited by toxin, although germination percentages were not. Seedling lengths and weights were not significantly different.

Discussion

Early infection of field-grown sh2 seeds, primarily by Fusarium moniliforme, can be attributed to several factors. In Chapter II, rainfall in late spring and early summer was shown to delay, to some extent, maturation of field-grown kernels of sh2 and su. The higher moisture content of sh2 would leave the kernels vulnerable to fungal infection for a longer period of time (Chapter II). Ear and kernel rots can cause

Teble 4-6 Anelysis of leechete from infected (I) and non-infected (NI) mature greenhouse-grown sh2 end su seeds

Type	Leechete	
	Conductivity (µmhos/g seed)	Tetal carbohydretes (mg glucose/g seed)
sh2 I	64.2a[z]	7.6b
sh2 NI	70.1e	15.9e
su I	19.8b	2 4c
su NI	21.3b	3.3c

[z]Neen separetion in celumns by Duncen's multiple renge test, 5% level

Table 4-7. Respiration rates of infected (I) and noninfected (NI) mature greenhouse-grown sh2 and su seeds

Type	Imbibition time (hr)				
	4	12	24	36	48
	($\mu\ell$ O_2/hr/g dry wt)				
sh2 I	106e[z]	151e	221a	252ab	924a
sh2 NI	93a	137a	295a	303a	511b
su I	30b	36b	120b	182ab	317c
su NI	32b	41b	117b	146b	221d

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

Table 4-8. Growth of excised embryos of noninfected mature greenhouse-grown sh2 and su seeds on sucrose-agar media with or without toxin

Type	Treatment[z]	Germination (%)	Abnormal seedlings (%)	Radicle length (cm)	Seedling length (cm)	Seedling wt	
						Fresh (mg)	Dry (mg)
sh2	H_2O-SA	83a[y]	10a	3.6a	6.4a	306a	47a
	H_2O-Toxin	87a	15a	2.9b	6.1a	323a	46a
	Toxin-Toxin	81a	13a	2.8b	5.7a	310a	47a
su	H_2O-SA	98a[y]	10a	3.9a	7.3a	271a	41a
	H_2O-Toxin	90a	15a	2.7b	6.7a	284a	40a
	Toxin-Toxin	92a	26a	2.7b	7.7a	296a	38a

[z]Seeds imbibed in H_2O and embryos plated on sucrose-agar, imbibed in H_2O and plated on sucrose-agar + toxin, or imbibed in toxin and plated on sucrose-agar + toxin.

[y]Mean separation in columns by Duncan's multiple range test, 5% level.

considerable damage when rainfall is above normal from silking to harvest, as in Florida. Ears maturing in a downward position (su) usually have less rot than ears maturing upright, such as sh2 (Shurtleff, 1977)

Large populations of corn earworm (Heliothis zea) and the fall armyworm (Spodoptera frugiperda) damaged commercial ears in Florida, even under intensive spray programs (Mitchell, 1978). Insect damage can increase incidence of stalk and ear rots by Fusarium moniliforme (Christensen and Wilcoxson, 1966, Koehler, 1960, Shurtleff, 1977, Smeltzer, 1959). In the present study, kernel infection by F. moniliforme in the tip half of ears was more pronounced, but high levels of infection were also noted from the butt half. King (1981) isolated more F. moniliforme from the tip than butt halves of ears grown in Mississippi. Infection of silks (Koehler, 1942) and initial earworm damage at the tips of ears (Mitchell, 1978) may be responsible for entrance of F. moniliforme through the ear tips. Once inside, infection can spread rapidly throughout the ear (Koehler, 1942) and also be aided by fall armyworms burrowing into the sides of ears (Mitchell, 1978).

Greenhouse-grown plants of sh2 and su benefited from an optimum environment, no competition between plants, and no insects or field fungi. High humidity in the greenhouse during seed maturation was primarily responsible for the Penicillium infection that occurred. Heavier and more vigorous seeds, especially of mature sh2, were therefore produced in the greenhouse (Chapter II).

Ears of sh2 and su inoculated with F. moniliforme 10 days post-pollination and harvested 38 to 46 days post-pollination had the greatest amounts of ear rot and seed infection. Generally, inoculated

ears of both genotypes with the greatest degree of ear rot tended to have the highest number of asymptomatically infected seeds. Workers previously found that ear infection was greatest when inoculation occurred 4 to 14 days past pollination, and least 21 or more days past pollination (Ullstrup, 1970, Warren, 1978). The lower ear rot and asymptomatic seed infection produced in sh2 and su ears inoculated 20 days post-pollination may be due to a shorter time for disease development, lower moisture content of silks and kernels, and/or kernels with more resistence due to structural changes. However, sh2 ears rotted to some extent within 10 days after inoculation while su ears did not. This difference suggested that the high-sugar genotype (sh2) was more susceptible to infection with F. moniliforme, as shown in high-lysine corn genotypes, such as opaque-2 (Gulya et al., 1980, Loesch et al., 1976, Ullstrup, 1971, Warren, 1978). Genotypic differences in susceptibility have also been reported with inbreds (Smith and Madson, 1949, Warren, 1978) and their hybrids (King and Scott, 1981).

An examination of seed surface morphology revealed no large cracks in the pericarp of sh2, regardless of maturity (Chapter III). The seed surface was fairly smooth and did not appear likely to be able to trap spores of F. moniliforme. Inglis (1980) found F. moniliforme spores trapped in natural crevices in the rough surface of asparagus seeds. The pathogen did not appear to be primarily a surface contaminant occurring in cracks and natural openings in the pericarp, as suggested by El-Helelgi et al. (1981). Although hyphae were able to enter through very small cracks in the pericarp of sh2 seeds, direct penetration was achieved by formation of appressoria. Upon penetration of the pericarp, hyphae were able to grow and sporulate in open areas between

the pericarp and aleurone layer. In sh2 seeds, the pericarp tended to pull away from the aleurone layer during maturation to form small pockets. Once in this pocket, colonization of the aleurone layer could take place with subsequent deeper penetration into endosperm and embryo. Johnson (1935) demonstrated that the aleurone layer would support a heavy growth of fungi when the seed coat was penetrated. On the other hand, Jones et al. (1972) reported that the aleurone layer did not allow further penetration of Sclerospore sorghi into corn seeds.

Fusarium moniliforme was present in tip caps, endosperms, and embryos of mature sh2 and su seeds, whether grown in the field or grown and inoculated in the greenhouse. Deep-seated infections were not obtained in less mature seeds of field-grown sh2 and su. Isolation of F. moniliforme from inner tissues of seeds is not uncommon (Mathur et al., 1975; Salama and Mishricky, 1973). Early inoculation of sorghum ears with F. moniliforme produced deeply infected kernels (Castor and Frederiksen, 1981). These kernels matured earlier and were smaller than normal kernels. Reduction in seed size and yield due to infection with F. moniliforme had previously been reported in sorghum (Castor and Frederiksen, 1980). Earlier field infection of sh2 kernels compared with su can produce deeply established F. moniliforme that could then reduce seed size and affect seed maturity.

Isolation of F. moniliforme from the tip caps of infected sh2 and su seeds indicated that the pathogen entered the seeds through the tip caps. Many ear and kernel rots begin at the tip end of ears (Shurtleff, 1977). Earworm damage would occur initially at the ear tip with fungi gaining access to the cob at this point. From here, the pathogens would

move through the cob and infect the kernels through the tip caps (Lawrence et al., 1981; Salama and Mishricky, 1973)

Infection by F. moniliforme affected the viability and vigor of mature greenhouse-grown sh2 and su seeds. Root and shoot growth of sh2 was significantly reduced when seeds were germinated under optimum conditions. Infected su seeds produced more abnormal seedlings than noninfected seeds. Fusarium moniliforme on or in the seed was the only noncontrolled factor influencing germination and seedling growth under optimum conditions. Mathur et al. (1975) determined that F. moniliforme affected both germination and seedling growth of sorghum when germinated on blotters or in soil. Root growth of corn seedlings was inhibited when seeds were inoculated with F. moniliforme (Fntrell and Kilgore, 1969). Abnormal germination in corn seeds of an inbred line was caused by infection with Fusarium and Penicillium spp. (Kushihiki et al., 1976). When sh2 seeds were placed in cold soil, germination and emergence of infected seeds were significantly less than noninfected ones, because seeds and seedlings weakened by infection may have been killed under these conditions.

Biochemical vigor measurements of infected and noninfected seeds were not as definitive as physiological vigor measurements. Seed leakage, as represented by conductivity and soluble carbohydrates, was actually greater in noninfected than infected seeds of both sh2 and su. Infected seeds would be expected to leak more, as is the case with low-vigor and dead seeds (Perry and Harrison, 1970). An explanation for this occurrence could be that bacteria and fungi associated with the soaking seeds multiplied rapidly within 24 hours and utilized some of

the leached substances to grow and reproduce (G. E. Harman, personal communication)

Fusarium moniliforme may not be effecting the seed, physiologically or biochemically, until the seedling stage. Respiration rates of infected and noninfected seeds of sh2 and sn did not significantly differ until 48 hours of imbibition. By this time, infected seeds respired at a higher rate than noninfected seeds, and radicle emergence had already occurred in all seeds. Evidently, fungal deterioration of embryos of infected seeds had not begun prior to the seedling stage. These results are in contrast to the work of Herman and Drury (1973), who found that respiration of axes from aged peas infected with Aspergillus ruber increased much less than respiration of axes from noninfected aged peas after imbibition. They also indicated that respiration of testea and cotyledons of unimbibed infected pea seeds was primarily fungal. Fusarium moniliforme may be interfering with the seed and seedling due to its deep-seated location within infected sh2 and su seeds. The increased respiration rate for infected seeds after 48 hours of imbibition may be due to the fungus and not the seed or seedling. Harper and Lynch (1981) noted that growth of seed-borne fungi under the husk of barley seeds increased during imbibition and continued when the seed was fully imbibed, resulting in poor seed germination. They suggested that competition for oxygen between barley seeds and seed-borne fungi located beneath the husk was probably the mechanism by which germination was suppressed.

A heat-stable, water-soluble toxin from F. moniliforme adversely effected root growth of noninfected excised sh2 and su embryos. This inhibition of root growth was partially overcome in su embryos by an

increased amount of shoot growth. Fusarium moniliforme toxin has previously been shown to decrease root growth of whole corn seedlings (Futrell and Kilgore, 1965; Scott and Futrell, 1970), and caused abnormality and reduced growth of noninfected corn embryos (Brodnik, 1975). Unlike toxins produced by Helminthosporium maydis (Gregory et al., 1980) and Aspergillus ruber (Harman and Nash, 1972). Fusarium moniliforme toxin did not appear to act until after radicle emergence. At this point, the seedling may be effected by fungal growth and colonization, as well as by toxin.

In the present studies, the environment during seed production influenced sh2 viability and vigor (Chapter II). The environment of the greenhouse allowed optimum development of sh2 seeds, as noted in the vigor tests. However, the difference in viability and vigor of sh2 seeds produced in the field and greenhouse was primarily due to infection by Fusarium moniliforme during early stages of development. The association between earworm damage and the pathogen may play an important role. Earworms may preferentially attack sh2 ears, possibly due to the higher sugar content of the kernels. Thus, the pathogen could become established within sh2 ears at an earlier developmental period than within su ears. The stressful environment of the field may not allow sh2 plants to overcome this infection, possibly resulting in smaller, and less vigorous, infected seeds at maturity.

Various factors influenced infection during seed development and maturation of sh2 which ultimately effected seed viability and vigor. Infection during immature stages of development allowed F. moniliforme to become located deep within the seed by maturity. The pathogen invaded the ear while sugar levels were still fairly high in the kernels,

providing an ideal substrate for further growth (Chapter III). Establishment of the fungus was further enhanced by the continuous upright position of sh2 ears and high kernel moisture (Chapter II), which prolonged the period of susceptibility to infection. Additionally, the structure of immature sh2 seeds was not as protective as mature seeds (Chapter III). Until the kernels start to rapidly lose moisture, F. moniliforme has greater accessibility into internal tissues. The time and location of infection by F. moniliforme indicated that delayed development and maturation of sh2 seeds, as demonstrated in Chapter II, is the key to pathogenic susceptibility.

The effect of F. moniliforme on sh2 seeds became most noticeable during germination under stress conditions. The close association of the pathogen with the seed could produce seed rot and seedlings that were later overcame by the fungus. The production of toxic substances that inhibit seedling root growth was also helped by the location of F. moniliforme within the seed. The higher levels of leakage from sh2 than from su seeds could stimulate soil-borne, as well as seed-borne pathogens, to attack the seeds during germination. However, in mature sh2 seeds, this stimulation was not entirely due to sugar exudation (Chapter III).

Rapid imbibition rates of sh2 seeds could lead to seed injury, particularly in cold soils, thereby slowing germination and leaving the seeds even more vulnerable to pathogenic attack (Chapter III). The association of F. moniliforme with sh2 seeds during development significantly contributed to, but was not the primary reason for, the reduction in viability and vigor. The small endosperm size has been associated with slow seedling growth in sh2 (Wenn, 1980). Further work needs to be

done to investigate additional causes of poor seed vigor in sh2 Possible areas to explore should include the embryo-endosperm relationship and genetic inferiority of the embryo.

Summary

The association of Fusarium moniliforme during seed development of sh2 and su sweet corn was investigated utilizing ears naturally infected in the field or inoculated in the greenhouse. The effect of this pathogen on subsequent seed viability and vigor was determined using physiological and biochmical vigor tests. In addition, growth of excised embryos was measured after exposure to toxin of F. moniliforme.

Kernels of sh2 became heavily infected with F. moniliforme in the field earlier than su. Earworm damage and silk infection may facilitate entrence of the pathogen through the tip and of ears, with further spread through the cob likely. Mature ears of both genotypes inoculated 10 days post-pollination with F. moniliforme hed high levels of rot and infection, indicating that ears exposed the longest time to F. moniliforme suffered the most damage. Asymptomatically infected seeds of sh2 and su had the highest percenteges of infection when teken from inoculeted ears with the greatest degree of rot.

Although no differences were noted in seed surface morphology, F. moniliforme appeared to infect mature sh2 seeds either through very small cracks in the pericarp or by appressorial formation. Once inside, the fungus spread throughout pocket-like areas between the pericarp and aleurone layer. Further penetretion into the endosperm and embryo was noted with maturity. Entrance of the pathogen through the tip cap was also indicated. The early deep-seeted infection by F. moniliforme may

be responsible for smaller and less vigorous sh2 seeds, especially under conditions of germination stress.

Infection increased the number of abnormal su seedlings and reduced seedling growth of sh2 seeds germinated under optimum conditions. Cold soil conditions decreased the viability of infected sh2 seeds and greatly reduced growth rates from optimum germination conditions. Leakage was greater from noninfected than infected seeds of both genotypes. Respiration rates were not significantly different between infected and noninfected seeds of sh2 and su until 48 hours of imbibition, at which time infected seeds respired significantly more than noninfected seeds. Radicle emergence had occurred in all seeds by this time. When excised embryos of sh2 and su were exposed to *F. moniliforme* toxin, radicle growth was inhibited. From this data, the pathogen appeared to exert the greatest influence during the seedling stage, despite its deep-seated location. Poor seed vigor of sh2 was related to infection with *F. moniliforme*, but this was not the primary factor involved for the reduction in vigor.

APPENDIX

Table A-1 Weight of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30° C

Type	Days post-pollination							
	18	22	26	30	34	38	42	46
	(mg/seed)							
sh2 F	34d[z]	62d	79d	97d	102d	105c	115c	116b
sh2 GH	65a	92b	114b	123c	130c	121b	131b	124b
su F	46c	73c	106c	137b	168b	198a	202a	213a
su GH	57b	89a	131a	148a	180a	198a	193a	211a

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

Table A-2 Seedling weight of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and germinated under optimum conditions

Type	Days post-pollination							
	18	22	26	30	34	38	42	46
	Seedling fresh weight (mg)							
sh2 F	246b[z]	399b	435b	445c	446d	524c	463d	451c
sh2 GH	387a	531a	622a	670b	755b	605b	697b	837a
su F	214b	327c	390b	496c	571c	591b	624c	683b
su GH	427a	576a	661a	861a	972a	953a	871a	860a
	Seedling dry weight (mg)							
sh2 F	28b	51d	63d	69d	77c	83c	86d	88d
sh2 GH	37a	66b	81c	84c	95b	94b	100c	118c
su F	23b	59c	88b	116a	137a	160a	173a	170a
su GH	36a	78a	95a	110b	141a	160a	153b	158b

[z]Mean separation in columns by Duncan's multiple range test, 5% level

Table A-3. Viability and vigor of field-grown sh2 and su seeds harvested at various stages of development as determined by a modified (MCT) and sail cold test (SCT)

Type	Days post-pollination							
	18	22	26	30	34	38	42	46
				Germination (%)				
sh2 MCT	45b[Z]	67b	61b	55c	66b	59b	63b	79b
sh2 SCT	5c	18c	16c	8d	15c	21c	23c	59c
su MCT	78a	100a	98a	98a	100a	93a	98a	97a
su SCT	3c	28c	72b	86b	96a	96a	97a	92a
				Abnormal seedlings (%)				
sh2 MCT	9b	6b	12b	9b	15a	21a	21a	18a
sh2 SCT	3b	13b	7b	3b	8a	5b	11a	14a
su MCT	29a	46a	36a	30a	15a	8ab	13a	12a
su SCT	0b	11b	21b	17ab	5a	11ab	15a	7a
				Radicle length (cm)				
sh2 MCT	6.5a	7.6b	8.0b	6.5b	5.6b	5.6c	5.1b	7.9b
sh2 SCT	1.7b	3.2c	4.9c	5.2b	2.6c	5.0c	3.2b	6.9b
su MCT	8.3a	10.1a	10.1a	9.0a	10.5a	12.8a	11.7a	11.5a
su SCT	1.2b	4.8c	7.6b	9.4a	10.9a	10.5b	11.1a	12.6a
				Seedling length (cm)				
sh2 MCT	9.7a	11.4ab	11.9a	9.4b	8.0c	7.5c	7.3c	10.7c
sh2 SCT	5.6b	10.7ab	11.2a	9.2b	6.3c	11.6b	7.0c	13.6bc
su MCT	11.8a	13.1a	12.8a	11.1b	13.2b	16.6a	15.2b	15.0b
su SCT	2.1c	9.0b	14.1a	17.0a	17.3a	17.1a	19.4a	22.3a

[Z]Mean separation in columns by Duncan's multiple range test, 5% level

Table A-4. Viability and vigor of field-grown sh2 and su seeds harvested at various stages of development as determined by an optimum germination (Opt) and accelerated aging test (AA)

Type	Days post-pollination							
	18	22	26	30	34	38	42	46
				Germination (%)				
sh2 AA	47c[z]	69b	35c	29b	11c	28c	35c	50c
sh2 Opt	74b	84a	74b	88a	88ab	77b	62b	91a
su AA	61bc	96a	98a	89a	83b	80b	63b	65b
su Opt	96a	96a	100a	100a	100a	96a	94a	95a
				Seedling length (cm)				
sh2 AA	15.2a	11.1c	10.7c	2.5c	1.8c	5.9c	6.3c	7.2c
sh2 Opt	15.9a	20.0ab	19.0b	17.6b	16.1b	17.8b	13.9b	17.4b
su AA	14.3a	21.1a	24.0a	25.7a	27.0a	24.6a	25.6a	27.1a
su Opt	13.2a	16.1b	16.1b	16.6b	17.4b	17.1b	17.8b	21.0b
				Seedling dry weight (mg)				
sh2 AA	34a	48a	57b	62b	60d	83b	90c	84b
sh2 Opt	28a	51a	63b	69b	77c	83b	86c	88b
su AA	35a	54a	87a	111a	124b	156a	159b	161a
su Opt	29a	59a	88a	116a	137a	160a	173a	170a
				Leachate conductivity (umhos/g seed)				
sh2 AA	171a	118a	162a	169a	176a	136a	130a	95a
sh2 Opt	170a	84b	80b	128b	82b	130a	85b	66b
su AA	115b	46c	23c	20c	16c	19b	13c	13c
su Opt	172a	79b	39c	29c	24c	18b	16c	14c

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

Table A-5. Time course of imbibition of mature field-grown (F) and greenhouse-grown (GH) sh2 and su seeds

Type	Imbibition time (hours)								
	1	2	4	8	16	24	36	48	60
	Rate (% increase fresh weight)								
sh2 F	14a[Z]	22a	32a	45a	63a	85a	97b	117b	132b
sh2 GH	10b	16b	25b	39b	60b	78b	102a	136a	153a
su F	7c	10c	15c	22c	31c	43c	51d	62d	73d
su GH	7c	10c	16c	22c	33c	43c	56c	70c	82c

[Z]Mean separation in columns by Duncan's multiple range test, 5% level.

Table A-6. Analysis of leachate of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development

Type	Days post-pollination							
	18	22	26	30	34	38	42	46
				Conductivity (μmhos/g seed)				
sh2 F	170a[z]	84b	80a	126a	82a	130a	85a	65a
sh2 GH	139b	58a	80a	80b	71a	59b	48b	42b
su F	172a	75b	39b	29d	24c	18c	19c	14c
su GH	123c	61c	40b	51c	42b	23c	23c	18c
				Potassium (ppm/g seed)				
sh2 F	41a	21b	17b	27a	18b	25a	19a	14a
sh2 GH	39ab	30a	23a	24b	21a	17b	15b	14a
su F	36b	13bc	9d	7d	6d	4d	5c	4b
su GH	31c	16c	13c	12c	9c	6c	6c	5b
				Total carbohydrates (mg glucose/g seed)				
sh2 F	17.2b	10.0b	9.8b	12.3b	6.7b	1.6b	1.4a	0.7a
sh2 GH	31.4a	20.3a	15.3a	16.8a	12.5a	3.6ab	2.6a	1.4a
su F	10.2c	5.4c	2.5c	1.1d	1.3c	1.0b	1.7a	0.6a
su GH	16.3b	9.7b	7.3b	8.2c	8.3b	5.4a	3.7a	2.2a
				Total sugars (mg/g seed)				
sh2 F	14.44a	5.36a	4.39a	2.97a	2.65a	1.14a	0.95a	1.14a
sh2 GH	1.39b	1.97b	1.77b	2.22a	0.96b	0.03a	0.02a	0a
su F	0.92bc	1.82b	1.19bc	0.08b	0.47b	0.05a	0.35a	0.20a
su GH	0c	0c	0c	0b	0b	0a	0a	0a

[z]Mean separation in columns by Duncan's multiple range test, 5% level

Table A-7. Total carbohydrates and starch contents of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30°C to 8% moisture

Type	Days post-pollination							
	18	22	26	30	34	38	42	46
				Total carbohydrates (% dry weight)				
sh2 F	39.7c[z]	44.7c	43.3c	38.1c	37.7b	39.3b	37.7c	40.0c
sh2 GH	41.4c	42.0c	38.5d	34.2d	34.6b	35.6b	34.6c	35.2d
su F	49.8b	56.4b	54.9b	53.5b	59.9a	64.0a	60.5a	61.3a
su GH	57.0a	62.6a	64.5a	61.0a	63.0a	61.5a	61.2b	57.0b
				Starch (% dry weight)				
sh2 F	14.9c	18.7b	16.8b	14.4c	15.9c	16.6c	17.2c	15.3c
sh2 GH	10.8d	11.0c	10.4c	10.6d	9.6d	10.3d	10.9d	9.0d
su F	25.5b	33.2a	35.2a	36.1a	36.3a	39.6a	42.3a	41.1a
su GH	28.3a	33.0a	35.6a	32.4b	32.4b	35.7b	31.5b	32.2b

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

Table A-9. Analysis of sugar content of field-grown (F) and greenhouse-grown (GH) sh2 and su seeds harvested at various stages of development and dried for 2 weeks at 30° C to 8% moisture

Type	Days post-pollination							
	18	22	26	30	34	38	42	46
				Fructose (mg/g dry weight)				
sh2 F	8.54a[2]	7.52a	7.46a	4.95a	3.83a	3.07a	3.54a	1.47a
sh2 GH	7.54a	7.79a	6.47b	2.92b	1.55b	0.39b	0.35b	0.14b
su F	4.62b	3.32b	3.20c	2.61b	2.21b	1.56b	1.47b	0.88ab
su GH	3.22c	2.16c	1.52d	1.07c	1.12b	1.06b	0.79b	0.66ab
				Glucose (mg/g dry weight)				
sh2 F	2.55b	2.00b	1.59b	1.20c	0.72c	1.44b	1.86b	0.68b
sh2 GH	1.77b	2.00b	1.12b	0.34c	0.14c	0c	0c	0b
su F	3.59a	6.98a	10.34a	11.00a	9.36a	6.75a	6.02a	3.38a
su GH	2.86b	2.41b	2.20a	2.73b	4.11b	1.53b	0.72c	0.52b
				Sucrose (mg/g dry weight)				
sh2 F	64.5a	62.3a	72.9a	47.1c	37.7c	38.0bc	36.4ab	36.5a
sh2 GH	53.6b	58.1a	68.4a	63.7a	55.0a	44.0a	41.1a	43.2a
su F	35.5c	37.0b	53.4b	56.1b	48.0b	42.4ab	41.2a	39.8a
su GH	34.5c	32.6b	29.4c	31.9d	41.2c	31.7c	31.4b	29.5b
				Total sugars (mg/g dry weight)				
sh2 F	75.6a	71.8a	82.0a	53.3b	42.3b	42.5b	41.8a	39.0a
sh2 GH	63.0b	67.9a	76.0a	66.9a	56.7a	44.4ab	41.5a	43.3a
su F	44.1c	47.3b	67.0b	69.6a	60.1a	50.7a	47.7a	44.1a
su GH	40.6c	37.1c	33.1c	35.7c	46.4b	34.2c	32.9b	30.7b

[2]Mean separation in columns by Duncan's multiple range test, 5% level.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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